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Ovine Phosphatic Urolithiasis

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OVINE PHOSPHATIC UROLITHIASIS

BY

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OVINE PHOSPHATIC UROLITHIASIS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Animal Science Department Date

OVINE PHOSPHATIC UROLITHIASIS
Abstract

Don H. Bushman

Under the supervision of Professor R. J. Emerick
and Professor L. B. Embry

A series of experiments was conducted using lambs and rats to determine the effect of various levels and sources of dietary phosphates on the incidence of phosphatic urinary calculi. Additional experiments were conducted to determine the degree of protection afforded sheep against urolithiasis by feeding various salts, and to determine the effect of these salts on excretion and retention of various ions. The lambs used in the sheep experiments were crossbred lambs weighing approximately 30 to 33 kg. They were fed a fattening ration consisting of ground shelled corn, alfalfa hay or silage (corn or sorghum) and soybean meal (approximately 11% crude protein in the diets).

Disodium phosphate, monosodium phosphate and sodium tripolyphosphate, fed at levels to provide 0.60% phosphorus in the total ration, resulted in a high (60 - 70%) incidence of urinary calculi with no difference between compounds. Increasing the level of dietary calcium from 0.31 to 0.58% with the sodium phosphates reduced, but did not completely prevent, the incidence of urinary calculi. Dicalcium phosphate fed at a level providing 0.58% calcium and 0.60% phosphorus in the ration did not result in the production of urinary calculi. Serum and urine phosphorus values were lowest for the control lambs and lambs fed dicalcium phosphate.

Subsequent experiments were conducted with rats to compare the relative availability of calcium in calcium carbonate and dicalcium phosphate, and phosphorus in disodium phosphate and dicalcium phosphate using weight gain, bone ash and net retention as criteria. Phosphorus appeared to be approximately one-third more available from disodium phosphate than from dicalcium phosphate, but no difference in calcium availability was observed.

Calcium carbonate, disodium phosphate and magnesium oxide were used in a factorially designed experiment involving 4 levels (0.37, 0.57, 0.77 and 1.27%) of calcium, 2 levels (0.25 and 0.55%) of phosphorus and 2 levels (0.18 and 0.38%) of magnesium to study the relationships of these minerals to calculi formation in sheep. Increasing the level of dietary phosphorus from 0.25 to 0.58% resulted in a significant increase in the incidence of urinary calculi (1.6 vs. 41.8% incidence). Increasing the level of dietary calcium from 0.37 to 0.77% or greater significantly lowered, but did not completely prevent, calculi formation. Feeding diets containing 0.38% magnesium resulted in a nonsignificant decrease in the incidence of calculi, comparable to the reduction afforded by an equal amount of calcium. Serum and urine phosphorus were reduced by increases in dietary calcium. A reduction in urine phosphorus, but an increase in serum phosphorus, resulted from an increase in dietary magnesium. While increased dietary magnesium, fed with the lower level of calcium, reduced urinary phosphorus to a greater extent than did an equal amount of calcium, it did not promote a correspondingly greater reduction in calculi.

To study the effect of various chlorides and calcium carbonate in the prevention of urolithiasis, lambs were fed a high-phosphorus (0.60%) basal ration known to be calculogenic. Either ammonium chloride, calcium chloride, sodium chloride or calcium carbonate was added at levels of 0.5 and 1.5% of the diet. The controls had a 50% incidence of calculi. Ammonium chloride and calcium chloride fed at a level of 1.5% of the diet reduced (4% vs. 50% incidence) the incidence of calculi. This was accompanied by a reduction in urine pH. None of the compounds appeared to affect feed consumption or weight gain. Ammonium chloride fed in a supplement, only partially mixed with the remainder of the ration to provide the equivalent of 1.5% in the total ration, lowered feed consumption and weight gain. In a similar experiment the treatments in addition to the control were: ammonium chloride, 1%; calcium chloride, 1%; potassium chloride, 1%; sodium chloride, 4%; and calcium carbonate, 2%. The percent incidence of urolithiasis for each treatment in the order listed was 50, 5, 16, 85, 35 and 30. In addition to lowering the incidence of urinary calculi, 1% of ammonium chloride also lowered urinary pH. Feed consumption and weight gain appeared to be reduced by the ammonium chloride in this experiment. Potassium chloride, in addition to increasing urinary calculi, resulted in a reduction in feed consumption and weight gain. Based on retention and excretion data, variations in excretion patterns of calcium, sodium, chloride or potassium without a concomitant reduction in urinary pH did not appear to play

a major role in calculi prevention.

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INTRODUCTION

Urinary calculi, urolithiasis or "water-belly" as it is sometimes referred to in livestock, is one of the most prevalent nutritional diseases common to man and animals. Among the first reports of calculi in livestock are those recorded by Youatt in 1893 in England (Beveridge, 1942). In the early 1900's the occurrence of urinary calculi was also reported in several other countries, including the United States, by Pontium and co-workers (Beveridge, 1942).

Two distinct types of calculi account for the majority of uroliths found in livestock. One is composed of various phosphates, such as calcium and magnesium ammonium phosphate, and is referred to as phosphatic urinary calculi or apatite calculi. The other type of stone is composed chiefly of silica and is referred to as siliceous calculi. In addition the occurrence of xanthin calculi in sheep has been reported in New Zealand (Easterfield et al., 1930; Easterfield and Bruce, 1930). However, the occurrence of xanthin calculi is not common in the United States. In humans approximately 90% of the urinary calculi are composed principally of calcium oxalate or phosphates, while the remaining 10% are chiefly ureates and cystic calculi (Prince and Scardino, 1960; Herring, 1962; Prien, 1963).

Extensive study of the various types of calculi, especially those composed mainly of phosphates, citrates, oxalates and silica, has been conducted with laboratory animals. Although the research documented in this thesis is concerned almost entirely with phosphatic urinary calculi in sheep, literature dealing with the other types of calculi

is discussed in the light of similarities and differences existing between various types.

The experiments reported herein were conducted to determine the causative factor(s) in the formation of phosphatic urolithiasis and to establish methods for its prevention in lambs.

REVIEW OF LITERATURE

Phosphatic Urinary Calculi

Elevated levels of blood and/or urine phosphorus resulting from either a high intake of phosphorus (Haag and Palmer, 1928; Taysom et al., 1951; Lindley et al., 1953; Elam et al., 1956, 1959; Emerick and Embry, 1963, 1964) or variations in phosphorus metabolism (Emerick et al., 1959; Packett and Hauschild, 1964) have been shown to be associated with phosphatic urolithiasis in sheep. Although Packett and Hauschild (1964) obtained a positive relationship between the gross occurrence of calculi and serum and urine phosphorus values, both calculi- and noncalculi-forming animals had received an equal amount of dietary phosphorus. Therefore, they concluded that their results appeared to be due to a difference in phosphorus metabolism between individual animals. Emerick and co-workers (1959) reported similar results in sheep that were subsequently afflicted with siliceous or phosphatic urinary calculi.

Dibasic sodium phosphate and dibasic potassium phosphate have been the most common phosphorus sources used to obtain high levels of dietary phosphorus. However, Elam and co-workers (1956) obtained a significant increase in the occurrence of calculi in lambs fed either potassium acid phosphate or a combination of phosphoric acid and potassium carbonate. A failure to increase the incidence of calculi by feeding a ration containing phosphoric acid alone was thought to be due to a lower urinary pH that phosphoric acid would be expected to promote. Emerick and Embry (1963), using disodium phosphate to

increase phosphorus levels, indicated that the maximum level of dietary phosphorus that sheep can tolerate and still remain free of urinary calculi is between 0.33 and 0.63%.

Phosphatic calculi are normally composed of calcium, magnesium and ammonium phosphates. A rare instance of zinc phosphate has been reported in a stone composed principally of apatite (Parsons, 1953). Lindley et al. (1953) presented data indicating that calcium is not a causative factor in calculi formation. Other studies have shown that increasing the calcium level of a ration high in phosphorus reduces the incidence of calculi (Schneider et al., 1952; Gill et al., 1959; Emerick and Embry, 1963, 1964). Although Schneider and co-workers (1952) obtained no change in calcium, magnesium or phosphorus concentration in either blood or urine through supplementation of these minerals, they obtained a reduction in the incidence of calculi by increasing the calcium-to-phosphorus ratio from 0.5:1.0 to 1.25:1.0. On the other hand, Lindley et al. (1953) reported that the addition of calcium and magnesium to the diet lowered serum phosphorus whether or not the diet was supplemented with phosphorus. Also, Gill and co-workers (1959) working with rats reported that high levels of dietary calcium, administered as calcium lactate, reduced urinary phosphorus excretion and the occurrence of calculi. They concluded that the lower urinary phosphorus values probably resulted from the formation of an insoluble calcium phosphate in the gut, thus, reducing the amount of phosphorus absorbed. These authors further concluded that the inhibition of calculi formation was due to the change in

urinary phosphorus concentration, and that stones will not form when the urinary phosphorus level falls below a certain critical level.

Johnson et al. (1940) and Lindley et al. (1953) presented data indicating that a high level of dietary magnesium is not a causative factor in calculi formation. However, Kunkel et al. (1961) reported a positive correlation between serum magnesium and the occurrence of calculi. This was explained on the basis of a decrease in urinary magnesium excretion induced by an excessive excretion of phosphorus, and not by an increased magnesium absorption. Packett and Hauschild (1963) found no correlation between serum magnesium and the incidence of calculi, and Johnson and co-workers (1940) were unable to produce calculi in lambs by elevating blood magnesium levels threefold.

Several workers (Orent et al., 1934; Greenberg et al., 1938; Gershoff and Andrus, 1961; Faragalla and Gershoff, 1963; Forbes, 1964; Bunce et al., 1965) have reported that a magnesium deficiency results in kidney calcification, and that this effect may be aggravated by increasing dietary phosphorus (Forbes, 1964; Bunce et al., 1965). Faragalla and Gershoff (1963) have implicated vitamin B_6 deficiency in addition to sulfur, phosphorus and magnesium levels in in the diets of rats with the formation of urinary calculi. Feeding a high-phosphorus, vitamin B_6 deficient diet resulted in stones composed of calcium and magnesium phosphate, as well as calcium oxalate. The implication of these factors in the production of calcium oxalate urolithiasis will be discussed later.

Thyroxine administration may prevent the kidney calcification

associated with a magnesium deficiency (Hellenstein et al., 1957; Gershoff et al., 1958; Forbes, 1965) or excess dietary phosphorus (Selye, 1958). Histological studies by Hess et al. (1959) showed that in magnesium deficient animals there is a swelling and an increased enzymatic activity in the proximal convoluted tubular mitochondria, followed by epithelial necrosis and the appearance of calcium deposits. Based on these observations by Hess and co-workers, Forbes (1965) expressed the opinion that thyroxine may exert its effect by preventing deleterious changes in the mitochondrial membrane rather than through a direct effect on calcification.

Diethylstilbestrol (DES) has also been implicated in the formation of urinary calculi in farm animals. Serveral authors (Jordan, 1953; Bell et al., 1954; Wilkinson et al., 1955; Udall and Jensen, 1958) have indicated that DES may be a predisposing agent in calculi formation. However, these workers used 15 and 30 mg. implants for lambs, these dosages being larger than those currently recommended for this species. On the other hand, McDonald and Eddings (1957) reported that DES lowered the incidence of calculi in rats. Emerick and Embry (1964) working with currently recommended levels of DES (3 mg. implants or 2 mg. per lamb daily in the feed) reported no significant effect of DES on calculi formation in wether or ewe lambs receiving either a control or calculogenic ration.

Calcium Citrate Calculi

The mechanism for the formation of citrate calculi, as well as other types of calculi, appears to be complicated and probably involves many factors. While Amberg and McClure (1917) showed several years ago that relatively large amounts of citrate may be present in the urine, several workers have demonstrated an inverse relationship between the level of citric acid excretion and the occurrence of citrate urolithiasis (Kissern and Locks, 1941; Scott et al., 1943; Yarbro, 1958a). Conway et al. (1949) attributed this decrease in urinary citrate excretion in calculi-forming patients to the presence of bacteria which are capable of splitting citrate. Yarbro (1958b) reported that citrate, at least at physiological levels, had only a minor effect upon calculi solubility in the urine. He suggested that the low citrate excretion maintained in some chronic urolith-forming patients is a symptom of a basic metabolic defect, and not the cause of calculi formation.

Data reported by Freeman and Chang (1950) indicate an association between hypercalcemia and the formation of citrate calculi. Morris and Steenbock (1951) reported that an excessively high calcium intake is not essential for the production of calculi when the phosphate intake is low. These authors produced a large incidence of calculi in rats with a normal calcium low phosphorus diet. Under normal conditions the intake of dietary phosphorus is sufficient to render excess calcium insoluble in the digestive tract.

Van Reen et al. (1959a) reported that rats receiving a diet

conducive to urolith formation excreted more calcium and citrate and less phosphorus than animals fed diets which did not lead to stone formation. Further, they concluded that differences in citrate excretion could not be explained on the basis of an altered capacity of kidney homogenates to synthesize or degrade citric acid.

When Morris and Steenbock (1951) increased the level of alkalinity in the diet by feeding sodium bicarbonate, or sodium citrate which is oxidized to carbonate, the frequency of calculi production was increased. On the other hand, it was entirely prevented by feeding adequate phosphorus. Analysis of the stones from rats receiving a potentially alkaline diet revealed that the calculi were not composed entirely of citrate, but also contained carbonate. From the small amount of citrate found in the stones, Morris and Steenbock concluded that the citrate ion is not a primary agent responsible for the formation of urolithiasis, but that the primary agent appeared to be an excess of absorbed calcium. Excess calcium when fed as the carbonate or as the salt of citric acid would induce an increase in urine pH and a subsequent precipitation of calcium citrate. When the intake of base exceeds the rats' capacity to produce citric acid, carbonates and phosphates may also be incorporated into the calculi. Other workers (Kuyper and Mattill, 1933; Schuck, 1934; Sherman et al., 1936; Orten and Smith, 1937; Smith and Orten, 1938; Yarbro, 1956) have also reported that the excretion of citrate was partially dependent upon urinary pH with consumption of an alkaline material increasing urinary citrate excretion, and consumption of acidic materials decreasing its

excretion.

Parathormone (Freeman and Chang, 1950) and estrogens (Shorr et al., 1942) have also been reported to influence citrate excretion. The latter has been suggested as one of the reasons for a higher level of citrate excretion and a lower incidence of calculi in women.

Increased levels of vitamin D have also been shown to increase the blood and urine levels of citrate (Bellin and Steenbock, 1952; Steenbock et al., 1951; Bellin et al., 1954). Bellin and co-workers (1954) reported that while vitamin D increased urinary citrate excretion in rats fed diets varying widely in mineral content, the largest percent increase in citrate was obtained with rations containing phosphorus. They suggested that the effect of vitamin D on citrate is due to an increase in citrate synthesis rather than a decrease in its destruction. Yarbro (1956) reported that physiological levels of vitamin D in the diet appeared to increase citrate excretion even on acid-producing diets such as those high in protein or supplemented with ammonium chloride.

Van Reen et al. (1959b) reported that the casein-mineral ratio of the diet was of considerable importance in the prevention of urolithiasis. Sager and Spargo (1955) also reported that adult male rats which were protein-depleted develop calcium citrate calculi on a normal-calcium, low-phosphorus ration after 6 days. Skeletal x-ray films, parathyroid measurements and balance studies indicated that the low-phosphorus ration increased calcium absorption from the intestine. Under conditions of hypoproteinemia, this resulted in an increase in

the nonprotein-bound calcium in the blood. They suggested that citrate is drawn from the metabolic processes, apparently as a compensatory mechanism, and combines with the excess calcium in the blood. The renal excretion of calcium and citrate are increased and the urinary calcium citrate tends to precipitate. These data, together with those of Van Reen et al. (1959b) implicate dietary protein level as well as calcium and phosphorus levels as a possible significant factor in citrate calculi production.

In addition, McChance et al. (1942) and Hall and Lehmann (1944) have presented evidence for an increased calcium absorption in the presence of elevated levels of dietary protein. Van Reen et al. (1959a) suggested that although their high-protein diets resulted in an increased calcium absorption, the extra calcium was retained because of the greater growth and lower citrate excretion of the rats on a high protein diet.

Oxalate Urinary Calculi

High levels of urinary oxalate are generally associated with the formation of calcium oxalate urinary calculi, and dietary studies have suggested that urinary oxalate excretion is reflected in part by oxalate intake (Dempsey et al., 1960). However, Archer et al. (1957) concluded that the relatively small and uniform amounts of oxalate present in most foodstuffs are unlikely to result in oxalate calculi deposition. High levels of urinary oxalate are more generally associated with metabolic disturbances. Among the more prevalent are those obtained by feeding a diet deficient in vitamin B_6 (Gershoff and Faragalla, 1959; Gershoff et al., 1959; Gershoff and Prien, 1960; Andrus et al., 1960; Faragalla and Gershoff, 1963). In addition, the administration of high levels of glycine (Andrus et al., 1960; Dempsey et al., 1960), ethylene glycol (Gershoff and Andrus, 1962) or tryptophan (Gershoff and Prien, 1960) in conjunction with a vitamin B_6 deficiency have been reported to increase the level of oxalate excretion with a subsequent increase in the occurrence of oxalate urinary calculi. Andrus et al. (1960) reported that while 3% glycine in a vitamin B_6 deficient diet increased oxalate excretion and calculi formation, this level of glycine administered to rats receiving an adequate level of vitamin B_6 resulted in no detectable alterations.

Miller et al. (1958), working with water solutions, urine and artificial urine samples, conducted several in vitro studies concerning factors influencing calcium oxalate solubility. They reported that creatine (87 mg. %), hippuric acid (70 mg. %) and pH

over the normal physiological range had no affect. A slight solubilizing effect was obtained by the use of urea (3 gm. %), sodium sulfate (266 gm. %) and sodium dihydrogen phosphate (172 mg. %), while magnesium chloride (32 mg. %), sodium chloride (2 mg. %) and citric acid (100 mg. %) markedly increased oxalate solubility. Light and Zinsser (1961) reported similar results with magnesium, phosphate, citrate and urea, with citrate giving the greatest protection from oxalate precipitation. Hammarsten (1956) reported that a pH of 5 to 6 was optimal for experimental production of oxalate calculi in rats.

Gershoff and Andrus (1961) reported that increasing the level of dietary magnesium from 0.04% to 0.4% of the diet resulted in a marked reduction in the deposition of oxalate in vitamin B_6 deficient rats, even though the rats remained hyperoxaluric. The authors attributed the protective effect of the magnesium to changes in the solvent characteristics of the urine. On the other hand, vitamin B_6 deficient rats exhibited a direct correlation between dietary calcium levels and the urinary deposition of oxalate with this effect being greatest for rats receiving a diet low in magnesium. Supplemental magnesium reduced the incidence of calculi resulting from ethylene glycol administration (Gershoff and Andrus, 1962).

More recently Faragalla and Gershoff (1963) reported a series of experiments in which they studied the effect of vitamin B_6, magnesium, sulfate and phosphorus on the excretion of urinary metabolites and the occurrence of urinary calculi in rats. When only the levels of vitamin B_6 and magnesium were altered, the occurrence of urinary calculi was

restricted to animals receiving a diet deficient in vitamin B_6 and low in magnesium (0.04% of the diet). Irrespective of the vitamin B_6 level, feeding a high level of magnesium (0.4% of the diet) resulted in a significant increase in the excretion of citrate and magnesium, and a decrease in the excretion of inorganic sulfates and phosphates. While variations in the level of dietary magnesium had no effect on oxalate excretion, a deficiency of vitamin B_6 resulted in an increase in oxalate excretion regardless of the level of dietary magnesium. These results agree with those discussed earlier.

Varying the level of phosphorus (0.20, 0.40 and 0.80%) and magnesium (0.04 and 0.4%) in conjunction with a vitamin B_6 deficiency resulted in urolith formation in rats receiving the higher level of dietary magnesium and either the low or high level of phosphorus. This was the first time these authors had produced calculi in rats receiving 0.4% dietary magnesium. In rats fed the low phosphorus diet the calculi were a mixture of monohydrate and dihydrate calcium oxalate. Stone formation was accompanied by a marked decrease in urinary oxalate and phosphate excretion. On the other hand, when the diet high in phosphorus was fed, the stones were composed of a mixture of calcium and magnesium phosphate and monohydrate oxalate. Furthermore, a high level of dietary phosphorus resulted in apatite nephrocalcinosis in rats receiving vitamin B_6 and 0.04% magnesium.

A deficiency of magnesium has previously been associated with calcification of soft tissues other than the kidneys in the guinea pig and cotton rat (Moore et al., 1938; Constant and Phillips, 1954;

House and Hogan, 1955). Feeding high phosphorus diets to guinea pigs also decreased the absorption of magnesium (O'Dell et al., 1957). Based on these observations Faragalla and Gershoff (1963) concluded that the magnesium requirements appeared to be increased in rats fed high phosphorus diets and resulted in the precipitation of calcium phosphate calculi.

Faragalla and Gershoff (1963) also reported that a low level of dietary sulfur was partially protective against renal calcium oxalate urolithiasis in vitamin B_6 deficient rats. Rats receiving the low sulfur diet excreted significantly less urinary oxalate, sulfate, phosphate and magnesium than rats receiving a normal level of sulfur.

Siliceous Calculi

The occurrence of siliceous urinary calculi has been reported in certain areas throughout the United States and Canada (Whiting et al., 1958; Forman et al., 1959), and occurs most frequently in sheep and cattle grazing the western ranges. In these areas the forages usually have a high silica content (Forman and Sauer, 1962). However, failure to produce siliceous calculi by the administration of high levels of inorganic silica in water and/or feed indicates that a high intake of silicon is not solely responsible as the causative factor (Whiting et al., 1958; Emerick et al., 1959; Forman and Sauer, 1962).

Several workers (Emerick et al., 1963; Settle and Sauer, 1960; Keeler and Lovelace, 1959) have shown that siliceous deposits in the kidney and urinary bladder occurred when animals were fed relatively small amounts (2 - 3% of the diet) of the organic silicon compound, tetraethylorthosilicate (TES). Data reported by Emerick et al. (1963) indicate that increasing levels of TES in the diet increases kidney damage and urinary calculi incidence. Rats given highly siliceous drinking water in addition to TES in the diet exhibited a higher incidence of kidney damage than was observed in TES-fed rats receiving silica-free water. However, the administration of highly siliceous water in the absence of TES apparently had no detrimental effect.

When silica was administered either orally or by injection, the silica concentration of the urine showed a marked increase, but only slight increases in the concentration of silica were noted in the blood (King et al., 1933; Sauer et al., 1959; Settle and Sauer, 1960)

indicating a low renal threshold for silicate. Forman and Sauer (1962) and Settle and Sauer (1960) indicated that the silica passes through the glomerular membrane in the monomeric state and polymerizes at levels exceeding saturation (approximately 180 ppm). They found some urine samples to contain quantities of silica far in excess of saturation. Emerick et al. (1963) obtained evidence of silica polymerization in the urine of rats at a concentration as low as 137 ppm of total silica. Iler (1955) reported the rate of polymerization of silicic acid in an aqueous system to be proportional to the square of the silica concentration.

Research reported to data has failed to reveal the causative agents of siliceous urolithiasis. Beeson et al. (1943) reported high levels of silica and magnesium in the urine of lambs. They discussed the relationship of silica and magnesium in light of previous reports that magnesium compounds are effective in reducing the amount of silica in aqueous solution. However, the calculi produced in their study were composed almost entirely of silica and contained only insignificant amounts of magnesium. Forman and Sauer (1962) and Forman et al. (1958) implicated urinary pH. These authors indicated that the occurrence of calculi in animals fed forages high in silica and low in cation (calcium, magnesium, sodium, and potassium) content was probably due to a low urinary pH. Contrary to this, Bezeau et al. (1961) concluded that an acidic urinary pH per se did not seem to be the causative factor of silica urolithiasis.

Matrix-Mineral Interrelationships in Urinary Calculi Formation

Although theories on stone formation concerning the inorganic components of urinary calculi differ quite widely, most authors agree that an organic matrix is present in all stones, and is essential to the formation of all types of calculi. The matrix, as defined by Finlayson et al. (1961) is "the non-dialyzable remainder of a stone after its crystal component has been dissolved with a mild solvent".

The fact that urinary calculi matrices are composed largely of carbohydrate and protein has been reported by various researchers (Boyce and Garvey, 1956; Boyce and King, 1959; Boyce, 1960; Cornelius and Moulton, 1960; Cornelius and Bishop, 1961; Finlayson et al., 1961). Boyce et al. (1958) reported that the matrix shows approximately the same chemical composition regardless of the type of calculi formed. However, McGaughey (1961) reported that the chemical make-up of matrix material in the mouse is considerably different from the organic matrix in calculi from humans or cattle. Cornelius and Bishop (1961) reported a close anatomical similarity in matrix-crystal interrelationships between phosphatic urolithiasis occurring in dog and man. However, in phosphatic calculi forming rapidly in ruminants, the matrix-crystal interrelationship was quite different.

The composition of matrix as reported by McGaughey (1961) was similar to Bence-Jones protein in regard to the content of phospholipids, cholesterol and carbohydrate. While it also contained similarly high serine levels, it deviated from Bence-Jones protein radically in regard to the content of other amino acids.

Boyce (1960) gave the approximate composition of the matrix as 65% protein, 14% glucide, 10% "bound water" and 12% "bound inorganic ash". Boyce also reported that the glucide part of the matrix contains glucose, galactose, mannose, rhamnose, fructose, deoxypentose and hexosamine. The bound ash was a mixture of inorganic substances including calcium and phosphorus even when the major crystalline component was cystine or uric acid. King and Boyce (1958) reported that the protein component of the matrix contained the following amino acid residues: a-alanine, glutamic acid, aspartic acid, serine, leucine, isoleucine, valine, phenylalanine, glycine, threonine, lysine, and proline arranged in descending order according to their intensity with ninhydrin after paper chromatography. There also appeared to be traces of tryptophan, tyrosine, methionine and arginine. These observations were confirmed microbiologically. In addition the authors consistently noted two unidentified ninhydrin positive spots.

X-ray diffraction data indicated that the amount of amorphous, protein material of the matrices varies to a large degree (Finlayson et al., 1961). This led the authors to make the suggestion that a coprecipitation of conjugated protein and inorganic salts occurred. Cornelius (1963), however, conversely reported that a constant occurrence of neutral glycoprotein complexes in urolith matrix suggested that certain specific urinary glycoprotein conjugates may be important in ion binding and/or coprecipitation. Cornelius and Moulton (1960) and Cornelius (1963), in histochemical examination of

the kidneys of sheep developing urinary calculi, reported an increase in periodic acid-Schiff (PAS) positive glycoprotein accumulation within the cells of the proximal and distal convuluted tubules and collecting ducts. Increased levels of PAS material were also observed in the lumina of the tubules and on the surface of epithelial cells of the renal pelvis and urinary bladder. King and Boyce (1957) earlier reported that the mucoprotein matrix of both calciferous urinary calculi and salivary gland calculi are PAS positive. King and Boyce further reported that these calculi were metachromatic with toluidine blue indicating a mucopolysaccharide, and led the authors to suggest the presence of both a neutral mucoprotein and an acid conjugated mucopolysaccharide. The association between the various components within the matrix is thought to be through a binding of calcium, magnesium, phosphates and hydroxyl ions (Boyce et al., 1958; Udall and Chow, 1963).

Udall and Jensen (1958) have postulated that urinary mucoprotein, as measured by protein-bound hexosamine, is a measure of the predisposition for the formation of calculi. They found that shifting steers from a ration high in alfalfa hay to a fattening ration increased the excretion of protein-bound hexosamine in the urine. This effect was also noted with animals fed rations containing three times the National Research Council (N.R.C.) requirements for calcium. However, increasing the level of phosphorus in the diet did not alter the level of protein-bound hexosamine.

Where and how these matrices are formed is still largely

unanswered, although several theories have been proposed. Grimes (1957) proposes that bone matrix may serve as the origin. Baker and Sison (1954) postulated that a depolymerized renal ground substance serves as a precursor. Engfeldt (1958) and Boyce et al. (1958) postulated the origin to be a secretory product of transitional or collecting tubular epithelium.

Boyce and King (1963) described one theoretical possibility for the origin and growth of calculi in the following manner. As the mitochondria in the epithelium of the proximal tubules become damaged and cease to function, there is a cessation of mitochondrial enzymes, and normal metabolism is thus altered. This results in an accumulation of tiny masses of mucoprotein, matrix precursor, within the cells. The masses of mucoprotein enlarge until they fill the entire cell causing it to rupture. The tubular lumen then becomes filled with material undergoing reorganization in the tubular fluid to form fibrils. As calcium adheres to the matrix, the spherule forms a spheralith which under proper conditions for crystallization develops into a calculus. This growth is primarily apatite deposition, and continued growth and development of the stone would depend upon favorable conditions within the urine.

Keeler (1963) presented a theoretical mechanism involving matrix material in the formation of siliceous urinary calculi (figure 1). Monomeric silicon under the influence of an alkaline pH will polymerize as silicic acid to form a $(silicon)_n$ sol. The reactions which occur beyond the formation of this sol depend upon the conditions

within the urine. In the presence of an acid pH, low protein content and a high silicon content, the sol may form a silicon gel and then an inorganic laminated material. However, an organic laminated material may form in the presence of high protein and low silicon content. A cycling of these conditions in the urine could thus lead to the formation of a typical laminar stone.

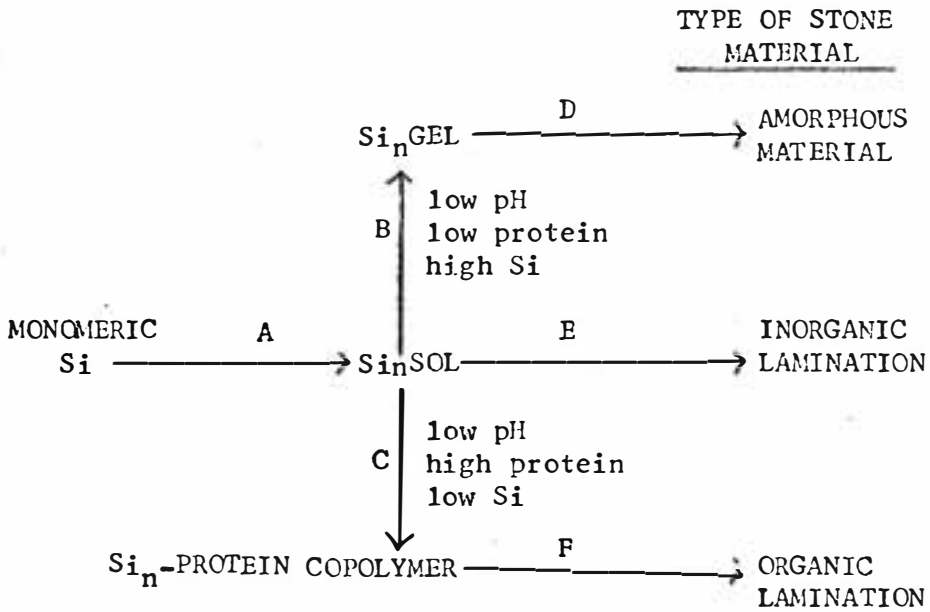

Figure 1. Possible mechanism for formation of bovine siliceous urolith material (Keeler, 1963).

The formation of these various stones are not all-or-none reactions, and various combinations of organic material may occur in laminations which are grossly inorganic and vice versa. The amorphous material may contain both silicon and protein. It appears that all three extremes of stone formation may result from a copolymerization forming a ($silicon_{n-1}$-$protein_{n-2}$) complex, the value of n-1 and n-2 depending upon the silicon and protein concentration in the

urine and urine pH.

Forman et al. (1959) supports the gelation theory of silicic acid based on the finding of structureless stones with an infinite number of undefinable nuclei, rather than a layered structure surrounding a central nucleus.

Finlayson et al. (1961) has pointed out that the matrix is only one aspect of urolith formation and that it should be viewed in context with the entire problem. They considered stone formation to be the summation of three factors:

Ex-solution of stone salts + matrix + time for growth = stone.

Figure 2 shows their proposed idea of stone formation and includes the place where many of the established and proposed factors are believed to exert their influence.

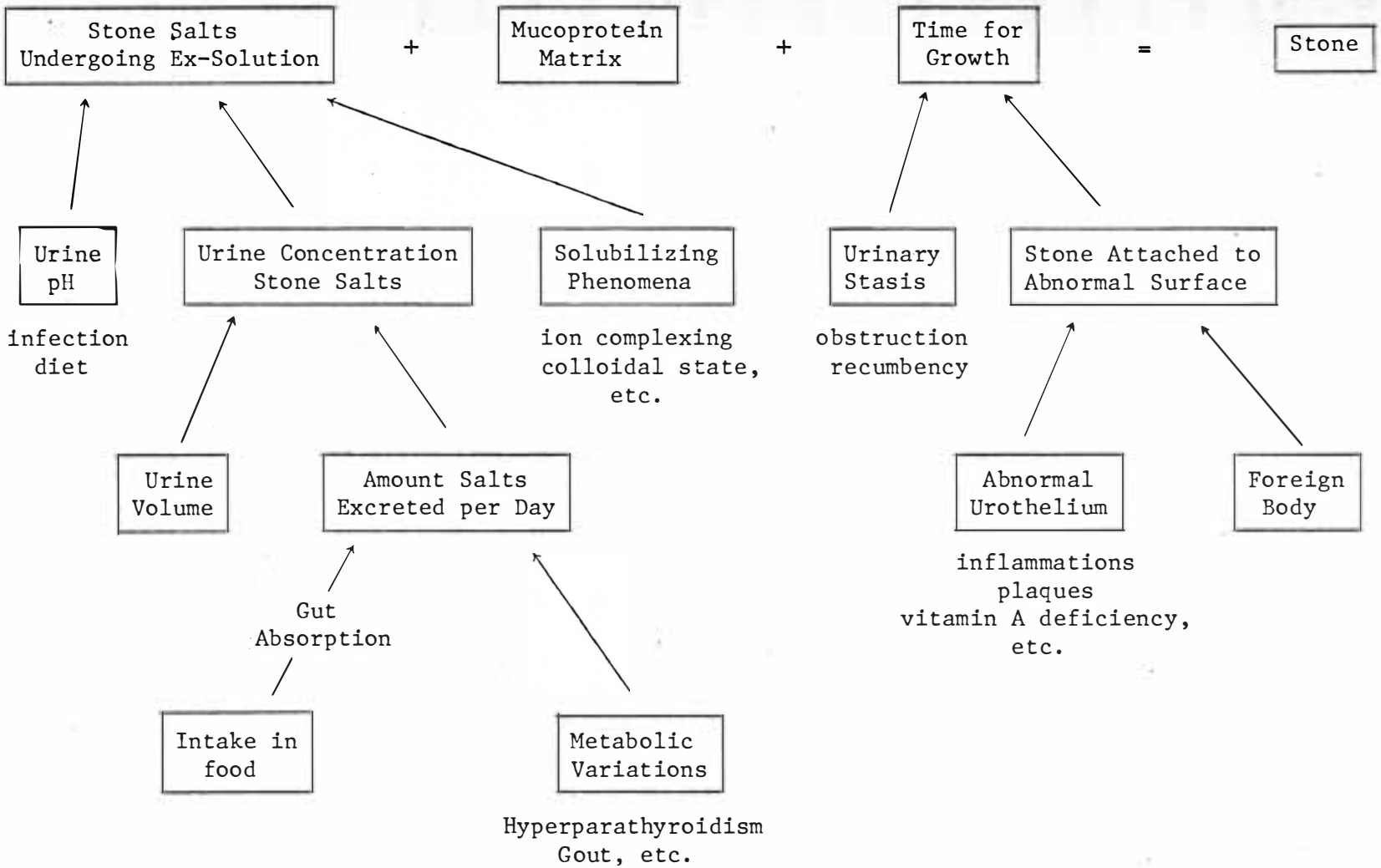

Figure 2. Schematic representation of stone formation showing where various factors, established or speculative, are thought to exert their influence (Finlayson et al., 1961).

Protective Factors

Protective Colloids. During the early 1950's the injection and implantation of hyaluronidase received attention as a preventative agent (Butt et al., 1952; Puntriano, 1954, 1955). Hyaluronidase, an enzyme which releases hyaluronic acid or one of its products, was thought to increase the action of protective colloids. Although some reports were favorable, very little research has been reported since 1955, and it has not been recommended for administration to livestock as a preventative measure in calculus formation.

Butt and co-workers (1952) working with humans and Puntriano (1954, 1955) working with siliceous calculi in sheep reported that hyaluronidase reduced the incidence of urinary calculi. Wiesel and Harlin (1953) reported that over a period of time hyaluronidase will lose its effectiveness through formation of antihyaluronidase antibodies. On the other hand, they reported that glucuronic acid, an end product of depolymerized hyaluronic acid, does not cause the production of antibodies, and can be used for prevention of calculi. Swingle and Marsh (1956) in addition to Butt and co-workers (1952) and Wiesel and Harlin (1953) have reported an inverse relationship between protective colloids and the incidence of urinary calculi. These authors have attributed the protective action of the colloids to changes in surface tension. Wiesel and Harlin reported that surface tension increased rapidly to its original level when glucuronic acid was withdrawn, but fell again when glucuronolactone was administered. Butt and co-workers (1952) reported that the colloids form a gel and

act as a dispersing agent, thus preventing electrolytes from crystallizing.

Contrary to the latter results, Culp and Flocks (1955) were unable to detect any effect of hyaluronidase upon the solubility of calcium phosphate, and several other workers (Ferguson, 1938; Boyce et al., 1954; Miller et al., 1958; Vermeulen et al., 1958) have failed to demonstrate any protective effect by a variety of colloids. Miller and co-workers concluded that the "super" solubility of calcium oxalate in urine could be explained by a "salting-in" effect of urinary electrolytes and the formation of complex ions. Vermeulen and co-workers reported that an unidentified solubilizing agent acted by enhancing "super" saturation, but this unidentified agent was found to be in the dialyzable portion of the urine and hence was not colloidal in nature.

Vermeulen and co-workers (1958) also reported that urinary electrolytes such as sodium, potassium, chloride and sulfate act to increase solubility by a nonspecific "salting-in" effect, but they play only a small role in permitting super saturation of calcium phosphate. Magnesium and citrate, on the other hand, by some unknown manner favored a temporary state of super saturation if present in sufficient concentration. While some of the workers mentioned previously have indicated a "super" solubility effect, Elliot (1957) reported that calcium phosphate calculi frequently develop when these salts are at less than saturation values.

Mineral Balance. A proper balance of calcium and phosphorus is

probably the most important means of preventing phosphatic and citrate urolithiasis. High levels of dietary phosphorus have been shown to promote phosphatic urinary calculi (Lindley et al., 1953; Elam et al., 1956, 1959; Emerick and Embry, 1963, 1964), and the studies of Schneider et al. (1952) and Emerick and Embry (1963, 1964) have shown that increasing the calcium level in high-phosphorus, calculogenic diets reduces the incidence of calculi. On the other hand, citrate urolithiasis is increased by elevated levels of dietary calcium (Freeman and Chang, 1950), or a low level of phosphorus (Morris and Steenbock, 1951).

The level of dietary magnesium has also been implicated with phosphorus in prevention and cause of urinary calculi. A deficiency of magnesium has been shown to induce calcification of kidneys (Orent et al., 1934; Greenberg et al., 1938; Forbes, 1964), and the inclusion of extra phosphorus in the diet increased the calcification (Forbes, 1964). Faragalla and Gershoff (1963) reported that increasing the dietary level of magnesium prevented the formation of oxalate calculi in vitamin B_6 deficient rats. However, under some conditions calculi were formed even if magnesium was high. A high-magnesium, high-phosphorus diet resulted in the formation of phosphatic uroliths, whereas a high-magnesium, low-phosphorus diet resulted in the formation of oxalate uroliths.

Variations in Urine pH. Newsom (1938) some years ago indicated that an alkaline urine is more conducive to phosphatic calculi formation than an acidic urine. More recently Elliot et al. (1961)

concluded from data obtained *in vitro* that the solubility of calcium phosphate, and thus the development of phosphatic urolithiasis, is influenced principally by urinary pH. Further, Elliot and co-workers concluded that the excretion of calcium and citrate are of little importance in preventing calculi formation. Morris and Steenbock (1951) also reported that feeding large amounts of alkaline supplements to rats aided in the formation of stones containing phosphates and carbonates, as well as citrates. Morris and Steenbock explained the occurrence of phosphates and carbonates in stones as being the result of the added alkali intake exceeding the capacity of the animals to synthesize citrate.

Boulet and Marier (1961) reported that calcium phosphate precipitation *in vitro* could be induced by adjusting the pH of solutions between 6 and 8 when the solutions contained between 2 and 24 moles per liter of calcium and phosphorus. With alkali additions below 1.40 to 1.45 equivalents per mole of total phosphorus, the precipitation occurred as dicalcium phosphate dihydrate. With additions of alkali above 1.40 to 1.45 equivalents per mole of phosphorus, the precipitation occurred in two distinct steps: (1) an immediate formation of a gelatinous precipitate, (2) followed by precipitation of a granular salt. The gelatinous, granular precipitates after washing gave an x-ray defraction pattern of hydroxyapatite, but it equilibrated with the supernatant as octacalcium phosphate.

Ammonium chloride is thought to reduce the incidence of calculi by lowering urinary pH (Vermeulen *et al.*, 1951; Leoschke and

Elvehjem, 1954; Crookshank et al., 1960). On the other hand, the drug diamox (acetazolamide) which increases urinary pH also increases the incidence of calculi (Presky et al., 1956; Shah et al., 1958; Mackenzie, 1960; Gill and Vermeulen, 1962). Gill and Vermeulen (1962) reported that diamox increased urinary ammonium excretion and decreased citrate excretion, resulting in an alkaline urine and a reduced magnesium-citrate complex. Both conditions favor the formation of magnesium ammonium phosphate calculi.

McDonald and Murphy (1959) and Murphy and Bradley (1961) suggested that a protective effect attributed to the feeding of hydrolized casein was due to a lowering of urinary pH. However, Murphy and Campbell (1961) reported that, while hydrolized casein reduced the size of stones formed in rats receiving increased dietary calcium and phosphorus, the control and casein treated rats had approximately the same pH. Thus, they ruled out a pH effect and postulated that the protective effect was due to a binding of calcium ions by the increased urinary amino acids. McGowen (1959) earlier reported that amino acids increased the solubility of calcium and magnesium phosphate. In both studies reported by Murphy and co-workers (Murphy and Bradley, 1961; Murphy and Campbell, 1961), urinary-bladder implants were used to induce calculi, and rats that produced an alkaline urine, a condition believed to be indicative of a urinary infection, were removed from the experiments.

Other workers (Bezeau et al., 1961; Udall, 1962; Udall and Chow, 1963) have reported that the formation of siliceous and phosphatic

calculi is not a simple function of urinary pH. Udall and co-workers used phosphoric acid and dibasic potassium carbonate to produce acidic and alkaline urines, respectively, and reported that phosphatic calculi occurred in lambs excreting either an alkaline or acidic urine.

Ammonium Chloride. Ammonium chloride has been reported by various workers to be effective in preventing calculi formation (Vermeulen et al., 1951; Leoschke and Elvehjem, 1954; Crookshank et al., 1960). Vermeulen and co-workers (1951) reported almost complete prevention of urolithiasis in rats receiving 1.5% of ammonium chloride in their diet. However, 1.5% of sodium carbonate appeared to increase the amount of calculi formed. Leoschke and Elvehjem (1954) and Crookshank and co-workers (1960) working with mink and steers, respectively, reported similar protective effects from feeding ammonium chloride.

In addition to preventing calculi formation, administration of 1.5% of ammonium chloride to rats that previously had been receiving a calculi-provoking diet appeared to aid in dissolving stones already formed (Vermeulen et al., 1951). In each instance, authors reporting on the use of ammonium chloride have attributed its effect on calculi formation to a reduction in urinary pH.

Sodium Chloride. Several workers have indicated that the addition of sodium chloride at appropriate levels to the ration will prevent calculi formation (Elam et al., 1957; Forman et al., 1958; Whiting et al., 1958; Forman and Sauer, 1962; Udall, 1959, 1962;

Udall and Chow, 1963; Bailey, 1967). Forman and co-workers, working with siliceous calculi, offered no explanation as to the mechanism of action involved in the prevention of calculi by sodium chloride. Bailey (1967) reported that feeding sodium chloride to steers significantly increased water intake, which was followed by a significant decrease in the concentration of silicic acid in the urine. Since silicic acid is known to polymerize when its concentration exceeds the saturation value, Bailey suggested that sodium chloride exerts its protective effect against siliceous calculi by lowering silicic acid concentration through an increase in urine volume. However, Udall and Chow (1963) working with phosphatic urinary calculi, reported that the protective action of sodium chloride is independent of its diuretic effect. They concluded that it is the chloride ion that interferes with urolith formation, and that the sodium ion has no inhibitory effect.

Udall and Chow hypothesized that the mechanism of action is manifested through a competition of the chloride ion for the anionic binding sites of the matrix material. They further suggested that the divalent cations calcium and magnesium are essential components of the matrix materials. Studies by Catchpole et al. (1956) and Carr (1956) concerning the binding of calcium and magnesium to various proteins supports this supposition.

Potassium Chloride. In addition to sodium chloride and ammonium chloride, potassium chloride has also been used for the prevention of phosphatic urolithiasis. Crookshank (1966) conducted an experiment

to compare the effect of several sodium and potassium salts upon the incidence of urolithiasis when fed in a known calculogenic ration. He reported that potassium salts were more effective than the corresponding sodium salts in reducing the incidence of phosphatic urinary calculi in lambs. While both 1% of sodium chloride and potassium chloride reduced the incidence of calculi, only potassium chloride resulted in a significant reduction.

Crookshank also reported that sodium bicarbonate produced a non-significant increase in the incidence of calculi, while potassium bicarbonate resulted in a highly significant decrease in calculi. However, the feeding of either sodium or potassium carbonate decreased calculi incidence.

Contrary to the results reported by Crookshank, Elam et al. (1956) reported that 1% of potassium carbonate increased the incidence of calculi. They further observed that, while phosphoric acid supplementation alone did not result in an increase in calculi formation, feeding phosphoric acid in conjunction with potassium carbonate resulted in a greater increase than was obtained with either material fed alone.

Urine Volume. Bailey (1967) has suggested that sodium chloride prevents siliceous urinary calculi by increasing urine volume, and lowering silicic acid concentration. Other workers (Udall and Chow, 1963; Lather et al., 1964), however, have reported no relationship between urine volume and the occurrence of calculi in sheep and humans, respectively. Elliot et al. (1961) expressed the idea that

dilution of urine may dilute protective substances more than ions involved in calculi formation and increase the tendency for mineral precipitation. In support of this, Robbins et al. (1965) noted an increase in urine volume in animals that were subsequently affected with urinary calculi.

EXPERIMENT 1. INCIDENCE OF URINARY CALCULI IN SHEEP AS AFFECTED BY VARIOUS DIETARY PHOSPHATES

High levels of dietary phosphorus have been shown to induce a high incidence of urinary calculi in sheep. Dibasic sodium phosphate and dibasic potassium phosphate have been the most commonly used sources of phosphorus employed in these studies.

Crookshank (1964) reported a lower incidence of urinary calculi in sheep from feeding dibasic potassium phosphate than from feeding the corresponding sodium salt. Elam et al. (1956) reported a 60% incidence of calculi when lambs were fed a high level of phosphorus as dibasic potassium phosphate and only a 5% incidence when fed an equal level of phosphorus as phosphoric acid.

This experiment was conducted to obtain additional information on the influence of variations in the chemical form of dietary phosphates on the incidence of urinary calculi in wether lambs.

Experimental

One hundred eighty crossbred wether lambs were used in this experiment conducted during an 84-day period in the late summer and early fall. The lambs averaging approximately 33 kg. were given 3 mg. diethylstilbestrol implants and were allotted on the basis of weight.

Treatments in the form of additions to the rations (table 1) consisted of the following: no added phosphorus (1 and 5), monosodium phosphate (2 and 6), disodium phosphate (3 and 7), sodium tripolyphosphate (4 and 8) and dicalcium phosphate (9). Each treatment

TABLE 1. RATION INGREDIENTS AND LEVELS OF CALCIUM AND PHOSPHORUS

	Treatment								
	1	2	3	4	5	6	7	8	9
Percent calcium[a]	0.31	0.31	0.31	0.31	0.58	0.58	0.58	0.58	0.58
Percent phosphorus[a]	0.25	0.60	0.60	0.60	0.25	0.60	0.60	0.60	0.60
Ingredient, %									
Silage[b]	66.70	66.70	66.70	66.70	66.70	66.70	66.70	66.70	66.70
Ground corn	28.26	27.23	26.95	27.23	27.62	26.57	26.40	26.57	27.01
Soybean oil meal	4.80	5.00	5.10	5.00	5.00	5.20	5.20	5.20	5.10
Ground limestone	0.24	0.23	0.23	0.23	0.68	0.68	0.68	0.68	0.05
Monosodium phosphate	----	0.84	----	----	----	0.85	----	----	----
Disodium phosphate	----	----	1.02	----	----	----	1.02	----	----
Sodium tripolyphosphate	----	----	----	0.84	----	----	----	0.84	----
Dicalcium phosphate	----	----	----	----	----	----	----	----	1.14

[a] Values for the total ration on a 10% moisture basis.

[b] Sorghum silage was fed during the first half of the experiment and corn silage during the second half. These silages contained 63.1% moisture.

was replicated with 10 lambs per replication. All phosphorus supplements provided an additional 0.7% phosphorus in the concentrate portion of the ration, equivalent to 0.35% phosphorus in the complete air-dry ration. They were fed with two levels of calcium (0.31 and 0.58% of the air-dry ration), using ground limestone as the supplementary source, with the exception of dicalcium phosphate which was fed only with the higher calcium level due to the calcium content of this phosphorus source.

Calcium and phosphorus supplements were supplied in the concentrate portion of the basal diet, which was fed at the ratio of one part of concentrate to two parts of silage (63.1% moisture). Sorghum silage was fed during the first half of the experiment. However, the supply was limited and corn silage was fed during the latter half. The rations were designed to contain 11% crude protein calculated on an air-dry basis and were full-fed once daily. Water and trace-mineralized salt were available free choice.

Feed samples were obtained periodically and analyzed for calcium, phosphorus and magnesium. Calcium and phosphorus were determined by the A.O.A.C. (1960) method. Magnesium was determined on the acid soluble portion of the feed ash by a modification of the procedure described by Kunkel et al. (1947) for urine.

A blood sample was obtained from each lamb after 3 weeks on experiment, and the serum was frozen and stored until analyzed. Overnight urine collections were also obtained from one lamb per treatment daily, alternating replications, until a sample had been obtained from

each lamb. Urine collections were made by use of an attached urinal fabricated from part of a rubber inner tube (figures 3 and 4). Serum calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method and urine calcium by McCudden's method (Hawk et al., 1954). Serum and urine phosphorus were determined by the method of Fiske and Subbarow (Hawk et al., 1954), and serum and urine magnesium by the method of Kunkel et al. (1947).

Lambs were observed for symptoms of urinary calculi throughout the experiment. Animals with apparent blockage of the urinary tract were slaughtered and the urinary tracts examined. At the termination of the experiment all remaining lambs were slaughtered and the urinary bladders and kidneys examined for calculi. Urinary stones were characterized according to type.

Data pertaining to average daily gain and feed consumption were calculated only for those lambs finishing the experiment. Since lambs with urinary calculi were slaughtered when symptoms were first observed, feed consumption data were corrected by subtracting an average value for each lamb removed.

Statistical analysis for comparisons among treatment means for calcium, phosphorus and magnesium levels in the serum and in urine was performed according to Duncan's multiple range test (Steel and Torrie, 1960). A statistical analysis of urinary calculi incidence among treatments was made by the chi-square method, and weight gain data by the method of least squares.

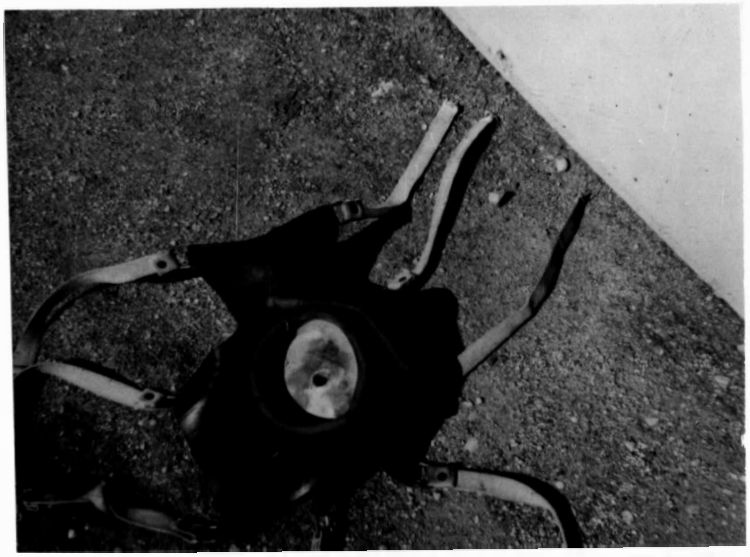

Figure 3. Urinal fabricated from part of a rubber inner tube and a copper collecting funnel (view from top).

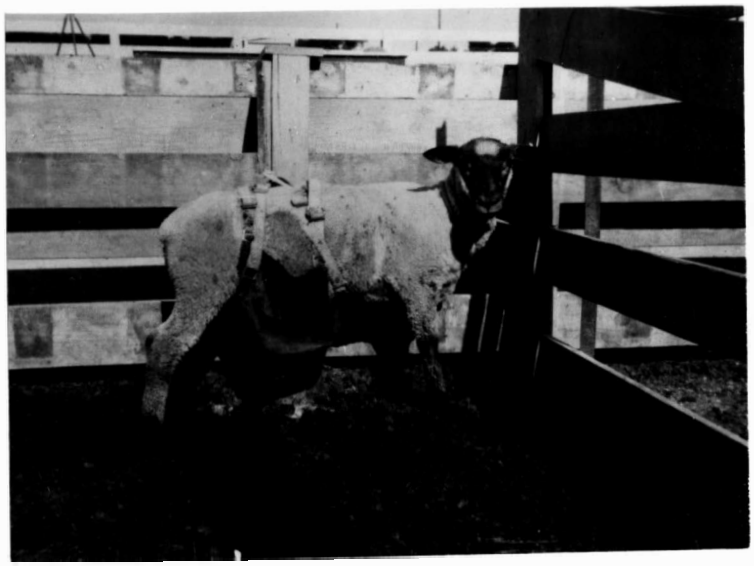

Figure 4. Urinal attached to a lamb.

Results and Discussion

Typical examples of lambs exhibiting symptoms of urinary calculi are shown in figures 5 and 6. Figures 7 through 9 exemplify the extensive damage to the urinary system often observed on autopsy, and figure 10 illustrates the various sizes and amounts of calculi recovered from the kidneys and urinary bladders.

The data from this experiment are reported in table 2. The high ratio of silage to concentrates fed during the first week, while the animals were gradually brought to full feed, resulted in a ratio slightly different from the one part concentrate to two parts silage fed during most of the experiment. Since two different silages were fed, there was some variation in the calcium and magnesium contents of the rations during the course of the experiment. Calcium and phosphorus contents of the rations as shown in table 2 are averages for the entire trial. When sorghum silage was fed, the low calcium rations contained 0.34% calcium and 0.21% magnesium. During the second half of the experiment when corn silage was fed, these rations contained 0.27% calcium and 0.19% magnesium. In addition to the variations caused by the source of silage, the source of dicalcium phosphate used in this experiment contributed a small amount of additional magnesium (0.04% on an air-dry basis) to the ration when this supplement was used.

The lowest levels of calcium and phosphorus (0.31 and 0.25%, respectively) used in this experiment are higher than the requirements of the National Research Council (N.R.C., 1964) for growing and

Figure 5. Lamb exhibiting signs of urinary calculi - note the stretched and strained condition of lamb in center of photograph.

Figure 6. Lamb suffering from pain of urinary-tract obstruction.

Figure 7. Urinary tract from a lamb with calculi showing the enlarged kidneys and distended urinary bladder.

Figure 8. Kidney with calculi (left) contrasted to a normal kidney (right).

Figure 9. Urinary calculi forced through the wall of the urethra.

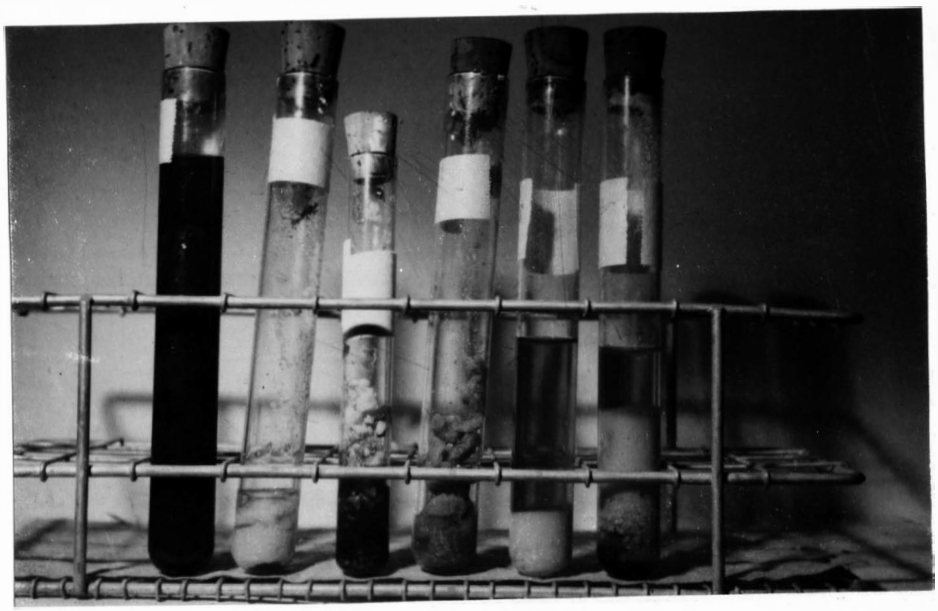

Figure 10. Variations in the consistency and amount of mineral deposits recovered from urinary tracts. Deposits may vary from sand-like consistency to large stones.

TABLE 2. EFFECT OF VARIOUS DIETARY PHOSPHATES IN SHEEP RATIONS

Treatment number	1	2	3	4	5	6	7	8	9
Supplemental phosphate sources	None	NaH_2PO_4	Na_2HPO_4	$Na_5P_3O_{10}$	None	NaH_2PO_4	Na_2HPO_4	$Na_5P_3O_{10}$	$CaHPO_4$
Calcium, %[a]	0.31	0.31	0.31	0.31	0.58	0.58	0.58	0.58	0.58
Phosphorus, %[a]	0.25	0.60	0.60	0.60	0.25	0.60	0.60	0.60	0.60
Number of lambs[b]	19	19	19	20	20	20	19	20	20
Average daily gain[c]	0.164	0.150	0.164	0.168	0.186	0.173	0.168	0.168	0.159
Daily ration, kg.[c]									
Concentrate mix	0.727	0.795	0.841	0.764	0.759	0.773	0.741	0.754	0.143
Silage[d]	1.52	1.66	1.74	1.59	1.58	1.61	1.54	1.58	1.63
Feed per 100 kg. gain, kg.[c]									
Concentrate mix	438	525	512	452	410	449	442	451	462
Silage[d]	917	1093	1059	940	857	937	918	943	966
Serum values, mg. per 100 ml.[e]									
Calcium	11.8^{f}	10.1^{g}	10.1^{g}	$10.7^{g,h,i}$	$11.2^{f,h,i}$	$10.7^{g,h,i}$	10.2^{g}	$10.4^{g,h}$	$11.5^{f,i}$
Phosphorus	7.0^{f}	$10.1^{g,h}$	11.1^{g}	10.8^{g}	6.9^{f}	$10.5^{g,h}$	$10.4^{g,h}$	$9.6^{g,h}$	$8.2^{f,h}$
Magnesium	$2.8^{f,g,h,i}$	$2.6^{f,g,h,k}$	3.8^{j}	3.2^{i}	$2.4^{f,g,k}$	2.2^{k}	$2.8^{g,h,i}$	$2.5^{f,g,k}$	$3.0^{h,i}$
Urine values, mg. per 100 ml.[l]									
Calcium	$1.2^{f,g}$	$0.8^{f,g}$	0.5^{g}	$0.6^{f,g}$	2.0^{f}	$0.9^{f,g}$	$0.8^{f,g}$	$0.9^{f,g}$	[illegible]
Phosphorus	3.4^{f}	45.3^{g}	41.1^{g}	47.5^{g}	3.1^{f}	$29.6^{g,h}$	$32.4^{g,h}$	$27.8^{g,h}$	[illegible]
Magnesium	54.8^{f}	$30.8^{g,h}$	27.5^{g}	$31.8^{g,h}$	$43.7^{f,g,h,i}$	$45.6^{f,h,i}$	$30.7^{g,h}$	$37.8^{g,h,i}$	$49.0^{f,i}$
Urine pH	8.74	8.48	8.75	8.54	8.80	8.68	8.80	8.70	8.54
Urinary calculi incidence									
Losses during experiment[m]	0	4	5	4	0	1	4	0	
Total incidence[n]	0^{f}	13^{g}	14^{g}	12^{g}	0^{f}	5^{h}	8^{h}	4^{h}	0^{f}

[a] Air-dry basis.

[b] Initially 20 lambs per treatment, but some losses occurred due to causes unrelated to the experiment.

[c] Calculated only for the animals surviving the entire experimental period.

[d] Wet basis.

[e] Due to losses before blood samples were collected, statistical analyses were calculated on the basis of 18 lambs per treatment.

[f,g,h,i,j,k] Treatment means on the same line bearing different superscript letters are significantly ($P < .05$) different.

[l] Due to losses before completion of urine collections, statistical analyses were conducted on the basis of 17 lambs per treatment.

[m] Death due to urine blockage, or slaughter when death appeared certain.

[n] Includes losses due to urine blockage and animals having urinary mineral deposits at termination of the experiment.

finishing lambs. Although there appears to be a trend toward higher rates of gain with the higher level of calcium, the differences were not significant.

Occurrence of urinary calculi was restricted to the groups receiving the higher (0.60%) level of phosphorus in the form of sodium phosphates. All calculi were of the phosphate type and contained both calcium and magnesium. There was no significant difference in the incidence of calculi among monosodium phosphate, disodium phosphate and sodium tripolyphosphate when fed at either level of calcium. In contrast with results obtained from use of the sodium phosphates, no urinary calculi occurred in the lambs receiving dicalcium phosphate as the supplementary source of phosphorus.

Increasing the calcium level from 0.31% to 0.58% gave partial protection, as evidenced by the lower incidence of urinary calculi with the higher level of dietary calcium. Urinary calculi incidence with 0.60% phosphorus and the 0.31 and 0.58% calcium levels, respectively, were 74 and 42% with disodium phosphate, 68 and 25% with monosodium phosphate and 60 and 20% with sodium tripolyphosphate. The lower incidence of urinary calculi with the higher calcium level was significant ($P<.05$) in all instances. The partial protection afforded by the higher calcium level is in agreement with the previous observations of Emerick and Embry (1963, 1964) and Schneider et al. (1952).

The absence of urinary calculi in the group of lambs fed dicalcium phosphate in this experiment cannot be explained on the basis of

the calcium level alone, since lambs fed equal levels of calcium but other sources of phosphorus developed calculi. The effect of sodium in this experiment was not determined. Crookshank (1966) reported that feeding dibasic sodium phosphate to sheep increased the incidence of urinary calculi more than the feeding of the corresponding potassium salt. The effect of magnesium, which appears to be contributed by some sources of dicalcium phosphate, and differences in the availability of calcium and phosphorus related to various phosphorus sources appear to warrant further investigation.

Diethylstilbestrol implants were used for all lambs in this experiment. Previous observations of Emerick and Embry (1964) have indicated that urinary calculi formation was not influenced by feeding approximately 2 mg. diethylstilbestrol per head daily or implanting with 3 mg. The absence of calculi when feeding the low level of phosphorus is in agreement with these previous observations.

Serum and urine phosphorus values appeared to be related to the level of phosphorus in the diet and to the incidence of urinary calculi. Lambs receiving treatments 1 and 5 (no added phosphorus) and having no calculi had significantly ($P < .05$) lower serum and urine phosphorus values than lambs in which urinary calculi occurred. Of the phosphorus-supplemented lambs, either with or without added calcium, those receiving dicalcium phosphate apparently had the lowest average serum and urine phosphorus values, but the difference was not significant when compared with groups receiving comparable calcium levels. Increasing the calcium level of the diet from 0.31% to 0.58%

tended to lower serum and urine phosphorus values (table 2). This effect was more pronounced in the case of urine phosphorus values; however, the differences were not significant. This effect of calcium is in agreement with data published previously (Emerick and Embry, 1963, 1964; Packett and Hauschild, 1963; Gill et al., 1959).

Values presented in table 2 indicate an inverse relationship between average serum and urine calcium levels and the incidence of urolithiasis. Groups in which no calculi occurred tended to have higher average serum and urine calcium values. Contrary to results reported by Kunkel et al. (1961), no apparent trend was found between serum magnesium and the incidence of urinary calculi in this experiment. Both serum and urinary magnesium were highly variable under the conditions of this study, and they support no definite conclusions concerning their relationship to urinary calculi formation. Packett and Hauschild (1964) reported that magnesium levels in the serum and urine were high in animals fed a calculi-provoking diet whether or not calculi were formed.

No difference was noted in urine pH values between treatment groups, and all values were alkaline, averaging about 8.7 (table 2). Previous work by Crookshank et al. (1960) and Newsom (1938) has indicated that alkaline urine is more conducive than acidic urine to calculi formation. Udall and Chow (1963), however, reported the formation of phosphatic uroliths in lambs excreting urine of either alkaline or acidic pH.

On the basis of data reported herein and results reported by

others (Emerick and Embry, 1963; Packett and Hauschild, 1963; Schneider et al., 1952; Taysom et al., 1951) serum and urinary phosphorus values appear to be a better indicator than corresponding calcium and magnesium values of the extent to which a dietary regimen may be expected to provoke phosphatic urinary calculi formation in sheep.

The results of this experiment show that lambs are less prone to urinary calculi formation when fed dicalcium phosphate than when fed ground limestone with either monosodium phosphate, disodium phosphate, or sodium tripolyphosphate at equivalent levels of calcium and phosphorus.

Summary

Wether lambs were used in nine treatment groups of 19 to 20 lambs and were fed a basal ration containing 0.25% phosphorus to which 0.35% additional phosphorus was added as monosodium phosphate, disodium phosphate, sodium tripolyphosphate or dicalcium phosphate to give 0.60% total phosphorus. Each ration was fed with two levels of calcium (0.31 and 0.58%) except dicalcium phosphate which was fed only at the higher calcium level.

No urinary calculi occurred in lambs receiving 0.25% phosphorus in diets containing either 0.31 or 0.58% calcium, or in lambs receiving dicalcium phosphate. The percent incidence of urinary calculi was 70, 65 and 60, respectively, for lambs receiving disodium phosphate, monosodium phosphate and sodium tripolyphosphate when these supplements were fed with 0.31% calcium. Increasing the calcium level to 0.58% significantly ($P<.05$) lowered, but did not prevent, the

incidence of urinary calculi.

Serum and urine phosphorus values were lowest in those groups where calculi were absent with the controls (0.25% phosphorus and 0.31 or 0.58% calcium) having significantly ($P < .05$) lower values than groups in which calculi were present. Corresponding values for calcium and magnesium were more variable and differences were generally not significant.

EXPERIMENT 2. RELATIONSHIPS INVOLVING DIETARY CALCIUM, PHOSPHORUS AND MAGNESIUM

In the first experiment, lambs receiving supplemental dicalcium phosphate had a significantly lower incidence of urolithiasis than lambs receiving comparable levels of calcium and phosphorus from various sodium phosphates and calcium carbonate. The source of dicalcium phosphate used was found to contain a significant quantity of magnesium. Further, contradictory results have been obtained concerning the relationship between magnesium and the incidence of urinary calculi. This experiment was conducted to determine the influence of supplemental magnesium on urinary calculi formation in lambs fed diets varying widely in calcium and phosphorus contents, and to determine if complete protection from phosphatic urinary calculi can be obtained through calcium supplementation.

A positive relationship between serum magnesium levels and the incidence of phosphatic urolithiasis was observed by Kunkel et al. (1961), and Crookshank and Robbins (1962) reported a reciprocal relationship between urinary excretion of magnesium and phosphorus in lambs. Johnson et al. (1940), however, reported that no calculi were produced in lambs when the level of plasma magnesium had been increased threefold. Packett and Hauschild (1964) reported that magnesium levels in the serum and urine were high in animals fed calculi-provoking diets whether or not calculi were formed.

Experimental

This experiment conducted over a 96-day period during late

summer and early fall, utilized 256 lambs in a 4 x 2 x 2 factorially designed experiment involving 4 levels of calcium, 2 levels of phosphorus and 2 levels of magnesium. The lambs, averaging 29 kg., were allotted to the 16 treatments on the basis of weight. All lambs were given 3 mg. diethylstilbestrol implants, a level which did not appear to influence urolithiasis in previous studies (Emerick and Embry, 1964).

The basal ration consisted of ground shelled corn, 77.4%; ground alfalfa hay, 20.0%; and soybean meal, 2.6%. The basal ration was shown by chemical analysis to contain 0.37% calcium, 0.25% phosphorus and 0.18% magnesium on an air-dry basis. Calcium and magnesium were determined on the acid-soluble portion of the feed ash by atomic absorption spectroscopy (Perkin-Elmer, 1964). Phosphorus was determined by the A.O.A.C. (1960) method.

Additions to the basal diet consisting of various levels of ground limestone, dibasic sodium phosphate and magnesium oxide were used to obtain the levels of calcium, phosphorus and magnesium in the 16 treatments as shown in table 3. The rations were designed to contain 11% crude protein (calculated) and were fed once daily in amounts so that feed was available at all times. Water and trace-mineralized salt were provided ad libitum.

A blood sample was obtained by jugular vein puncture from each lamb after 25 days on experiment and the serum was stored frozen until the analyses were performed. Overnight urine collections were obtained with collections being made from one lamb per treatment

daily until a sample had been obtained from each lamb. Urine collections were made by use of the apparatus described for experiment 1. Serum and urine calcium and magnesium were determined by atomic absorption spectroscopy (Perkin-Elmer, 1964), and serum and urine phosphorus by the method of Fiske and SubbaRow (Hawk et al., 1954).

The lambs were observed for symptoms of urolithiasis during the experiment and animals with apparent blockage of the urinary tract were slaughtered and the urinary tracts examined. At the termination of the experiment the remaining lambs were slaughtered and the urinary bladders and kidneys examined for calculi. All calculi were characterized according to type.

Statistical analysis of urolithiasis incidence among treatments was made by the chi-square method using separate orthoginal sets to test between calcium levels when the same level of calcium was used in two or more comparisons. Blood, urine and weight gain data were analyzed by the least squares method (Steel and Torrie, 1960). Data pertaining to average daily gain and feed consumption were calculated only for those lambs finishing the experiment. Feed consumption data were corrected by subtracting an average value for each lamb removed.

Results and Discussion

Data from this experiment are presented in table 3 and the analysis of variance is presented in table 4. A significant ($P<.01$) decrease in rate of gain was obtained with an increase in the level of either dietary phosphorus or magnesium. In addition, a significant ($P<.01$) interaction between calcium and phosphorus occurred. The

TABLE 3. EFFECT OF VARIOUS DIETARY CALCIUM, PHOSPHORUS AND MAGNESIUM LEVELS IN SHEEP RATIONS

	Dietary mineral variations															
Calcium,[1] %	0.37	0.37	0.57	0.57	0.77	0.77	1.27	1.27	0.37	0.37	0.57	0.57	0.77	0.77	1.27	1.27
Phosphorus,[1] %	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Magnesium,[1] %	0.18	0.38	0.18	0.38	0.18	0.38	0.18	0.38	0.18	0.38	0.18	0.38	0.18	0.38	0.18	0.38
No. of lambs[2]	14	16	14	16	16	15	16	16	15	16	13	16	16	16	16	14
Avg daily gain,[3] kg/day	0.214	0.236	0.240	0.210	0.225	0.207	0.219	0.212	0.205	0.157	0.219	0.193	0.205	0.195	0.242	0.229
Daily ration,[3] kg	1.261	1.356	1.365	1.315	1.370	1.306	1.306	1.270	1.288	1.293	1.293	1.143	1.279	1.256	1.388	1.343
Feed/gain[3]	5.87	5.74	5.68	6.26	6.10	6.32	5.95	5.98	6.29	8.22	5.91	5.92	6.24	6.43	5.74	5.87
Serum values, mg/100 ml																
Calcium	10.96	10.90	11.01	10.99	11.25	11.67	12.27	10.97	7.77	9.31	10.01	9.80	10.13	10.01	10.55	10.29
Phosphorus	6.86	7.52	7.16	6.94	6.56	7.18	7.08	7.06	9.27	12.07	8.61	9.51	9.94	9.14	8.37	9.62
Magnesium	2.75	3.66	2.51	3.27	2.71	3.10	2.81	3.07	2.98	3.90	3.03	3.74	2.84	2.53	2.57	3.53
Urine values, mg/100 ml																
Calcium	7.22	5.71	6.71	7.27	6.00	6.33	8.61	8.37	4.56	3.95	4.00	3.51	4.30	3.04	4.41	5.72
Phosphorus	3.70	2.30	2.32	1.45	1.90	1.69	2.50	2.94	67.88	33.58	49.89	26.31	30.40	20.50	6.09	7.76
Magnesium	107.26	174.20	88.65	196.65	163.15	207.36	141.90	210.96	71.87	110.41	61.16	103.89	104.65	106.07	122.67	125.54
Urine pH	8.5	8.5	8.6	8.5	8.7	8.6	8.5	8.6	8.0	8.4	8.4	8.4	8.4	8.5	8.8	8.5
Urinary calculi incidence																
In sheep lost during experiment[4]	0	0	0	0	1	0	0	0	5	4	2	4	1	3	0	0
Total incidence[5]	0	0	1	0	1	0	0	0	11	10	9	8	6	5	2	0

[1] Air-dry basis.
[2] Initially 16 lambs/treatment, but some losses occurred due to causes unrelated to the experiment.
[3] Calculated only for those animals surviving the entire experimental period.
[4] Death due to urine blockage, or slaughtered when death appeared certain.
[5] Includes losses due to urine blockage and animals having mineral deposits at termination of the experiment.

TABLE 4. ANALYSIS OF VARIANCE FOR AVERAGE DAILY WEIGHT GAIN AND FOR BLOOD AND URINE ANALYSIS

Source of variation	df	Mean squares: ADG	Effects on blood constituents: df	Ca	P	Mg	Effects on urine constituents: df	Ca	P	Mg
Ca	3	.0262	3	7.327**	11.18*	1.434**	3	40.580**	5,108*	2,479
P	1	.0656*	1	98.261**	391.96**	5.233**	1	482.602**	43,579**	417,219**
Mg	1	.0626*	1	3.669	25.03**	29.243**	1	3.367	5,152	56,692**
CaP	3	.0493**	3	0.315	8.50*	0.408	3	5.212	4,961*	2,665
CaMg	3	.0050	3	2.433	9.79	0.355	3	7.066	1,154	4,342
PMg	1	.0144	1	0.002	10.65	0.783	1	0.016	4,452**	406
Error	211[a]	.0112	232[b]	1.316	2.98	0.303	227[b]	9.847	469	3,508

[a] Calculated only for those animals surviving the entire experimental period.

[b] Due to losses before blood or urine samples were collected, statistical analysis could not be performed on an equal number of animals.

* $P < .05$.

** $P < .01$.

data indicate that this interaction was due to the apparent beneficial effect afforded by increased calcium levels used in conjunction with the higher phosphorus level. Since group feeding was used, no statistical analysis was applied to feed consumption or feed efficiency.

A 41.8% incidence of urolithiasis occurred in lambs fed the higher phosphorus level (0.55%). This is significantly ($P < .01$) higher than the 1.6% incidence occurring among lambs fed the rations containing 0.25% phosphorus. No examination was made for the presence of calculi at the beginning of the experiment, and it is possible that a low incidence existed initially such as that observed with the low-phosphorus rations.

Increases in the level of dietary calcium in conjunction with the higher level of dietary phosphorus were accompanied by decreases in the incidence of urolithiasis. The reductions were significant ($P < .05$) when calcium was increased from 0.37 to 0.77% or greater. However, complete protection did not appear to be provided even at the highest calcium level, with a 12.5% (2 cases out of 16 lambs) incidence occurring in the groups of lambs fed 1.27% of calcium and no added magnesium. It appears from these data that a calcium-to-phosphorus ratio exceeding 2:1 is required for optimal protection from urolithiasis in lambs fed high phosphorus rations. However, this ratio may vary for calcium and phosphorus sources differing in availability from those used in this experiment. These data concerning a reduction in the incidence of phosphatic urolithiasis by

dietary calcium are in agreement with data reported previously.

The addition of 0.20% magnesium to the diet in the form of magnesium oxide resulted in a nonsignificant decrease in the incidence of urolithiasis. However, with all levels of calcium used in this experiment, the added magnesium appeared to reduce the incidence of urolithiasis to an extent comparable to the reduction afforded by a similar amount of calcium. This is presented graphically in figure 11.

The mineral supplements used in this experiment appeared to have no affect upon urinary pH. All pH values were alkaline, averaging about 8.5.

The feeding of supplemental phosphorus, calcium or magnesium resulted in significantly ($P < .01$) higher levels of these respective minerals in both serum and urine. Nevertheless, an increase in dietary phosphorus significantly ($P < .01$) lowered serum and urinary calcium.

An increase in dietary magnesium contributed to a significant ($P < .01$) decrease in urinary phosphorus, but not serum phosphorus. This reduction in urinary phosphorus was most apparent when the 0.20% of supplemental magnesium was used in conjunction with the lower levels of calcium and absent when used with the highest level. The failure of dietary magnesium to exert an effect when used with the highest level of dietary calcium may be due to the relatively low level to which urine phosphorus had already been reduced by this level of calcium. However, there was a significant ($P < .01$)

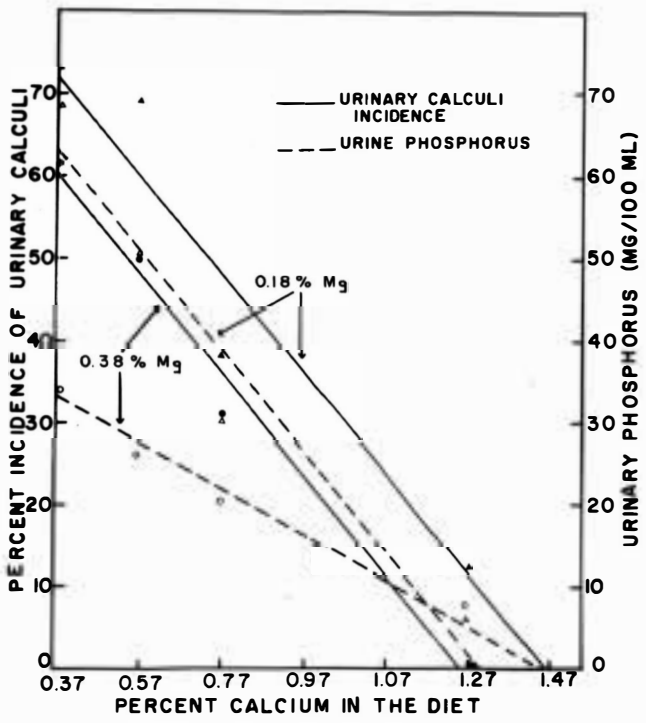

Figure 11. Incidence of urinary calculi and urinary phosphorus in lambs fed 0.55% phosphorus; ▲——▲ incidence of calculi when no supplemental magnesium was fed, ▲– – –▲ level of urine phosphorus when no supplemental magnesium was fed, o——o incidence of calculi when 0.20% supplemental magnesium was fed, o– – –o level of urine phosphorus when 0.20% supplemental magnesium was fed.

reduction in serum magnesium resulting from increases in dietary calcium which indicates a possible effect of calcium on the availability of magnesium. In support of the latter are results obtained with other species in which high levels of dietary calcium have been shown to increase the requirement for magnesium (O'Dell et al., 1960; McAleese and Forbes, 1961; Morris and O'Dell, 1963).

Contrary to the decrease in urinary phosphorus resulting from the feeding of supplemental magnesium, this treatment resulted in a significant ($P < .01$) increase in serum phosphorus. Increases in serum phosphorus, whether promoted by feeding supplemental magnesium or phosphorus, were accompanied by significant ($P < .01$) increases in serum magnesium. However, an inverse relationship between urinary phosphorus and magnesium was observed. Similar relationships between serum or urinary phosphorus and magnesium in lambs fed calculi-provoking diets have been reported previously (Robbins et al., 1965; Packett and Hauschild, 1964; Crookshank and Robbins, 1962). Furthermore, Clark et al. (1965) reported that high levels of magnesium in the diets of rats resulted in a more positive phosphorus balance when the diet was adequate in both calcium and phosphorus.

The relationship between average urinary phosphorus levels and the incidence of urolithiasis in groups of lambs with and without supplemental dietary magnesium is shown graphically in figure 11. Although 0.20% supplemental dietary magnesium, when fed with the lower levels of calcium, reduced urinary phosphorus to a larger degree than an equal amount of calcium, it did not exert a

correspondingly greater reduction in urolithiasis.

These data indicate that although a high urinary phosphorus level is probably the most important measurable factor associated with phosphatic urolithiasis, other factors may be involved. However, variations in magnesium metabolism appear to be of doubtful importance in the manifestation of this type of urolithiasis.

Summary

Wether lambs were used in a 4 x 2 x 2 factorially designed experiment involving 4 levels of dietary calcium, 2 levels of phosphorus and 2 levels of magnesium. Various levels of ground limestone, dibasic sodium phosphate and magnesium oxide were used to obtain 0.37, 0.57, 0.77 and 1.27% calcium, 0.25 and 0.55% phosphorus and 0.18 and 0.38% magnesium in the diet.

Lambs receiving the low level of dietary phosphorus had a 1.6% incidence of urinary calculi. Increasing the level of dietary phosphorus to 0.55% resulted in a 41.8% incidence of calculi, and this was accompanied by significantly higher serum and urine phosphorus values. Increasing the level of dietary calcium resulted in lower serum and urine phosphorus values and urinary calculi incidence. The higher level of dietary magnesium resulted in higher serum phosphorus but lower urinary phosphorus values. While dietary magnesium was more effective than an equal amount of calcium in reducing urinary phosphorus, it was no more effective in reducing urolithiasis. The data suggest that although a high urinary phosphorus level is a major causative factor of phosphatic urolithiasis, other factors are

probably involved even though variations in magnesium metabolism appear to be of doubtful importance.

EXPERIMENTS 3, 4 AND 5. CALCIUM AND PHOSPHORUS AVAILABILITY

Results of experiment 2 indicated that supplemental magnesium (magnesium oxide) appeared to offer no more protection against calculi formation than was provided by an equal amount of calcium supplied as calcium carbonate. Thus, the amount of magnesium present in the source of dicalcium phosphate used in experiment 1 could not have been sufficient to prevent calculi formation. Based on these observations, it appeared that differences in the calculi promoting effect of rations containing dicalcium phosphate or disodium phosphate and calcium carbonate might be due to differences in the availability of calcium and/or phosphorus. These experiments, therefore, were conducted to determine the relative availability of phosphorus in disodium phosphate and dicalcium phosphate, and calcium in calcium carbonate and dicalcium phosphate.

Experimental

Experiment 3. In this experiment, weanling male rats were used to compare the availability of calcium in dicalcium phosphate and calcium carbonate. The rats used in this and the subsequent experiments concerning calcium and phosphorus availability were of the Sprague-Dawley strain. Three rats from each of 15 litters (total 45 rats) were assigned to three groups providing a basis for littermate comparisons. Rats in treatment 1 received a low-calcium basal diet (table 5) containing 0.015% calcium and 0.38% phosphorus. Rats in treatments 2 and 3 received an additional 0.19% calcium from dicalcium phosphate and calcium carbonate, respectively. The phosphorus

TABLE 5. COMPOSITION OF RAT DIETS

	Experiment 3			Experiment 4		
	1	2	3	1	2	3
	%	%	%	%	%	%
Casein	20.0	20.0	20.0	----	----	----
Blood fibrin	----	----	----	20.0	20.0	20.0
Cerelose	70.3	70.0	69.8	69.9	69.4	68.9
Vegetable oil	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin mix[a]	2.0	2.0	2.0	2.0	2.0	2.0
Trace minerals[b,c]	1.2	1.2	1.2	1.2	1.2	1.2
Calcium carbonate[c]	----	----	0.449	1.374	0.729	1.374
Dicalcium phosphate[d]	----	0.859	----	----	1.111	----
Disodium phosphate[c]	0.248	0.248	0.723	----	----	0.916
Dipotassium phosphate[c]	1.288	0.723	1.288	----	----	----
Potassium chloride[c]	----	0.242	----	0.551	0.551	0.551

[a] Vitamin mix, concentration per kg.: vitamin A concentrate (200,000 USP units per gm.), 4.5 gm.; vitamin D concentrate (400,000 USP units per gm.), 0.25 gm.; alpha tocopherol, 5.0 gm.; ascorbic acid, 45.0 gm.; inositol, 5.0 mg.; choline, 75.0 gm.; riboflavin, 1.0 gm.; menadione, 2.25 gm.; p aminobenzoic acid, 5.0 gm.; niacin, 4.5 gm.; pyridoxine hydrochloride, 1.0 gm.; thiamine hydrochloride, 1.0 gm.; calcium pantothenate, 3.0 gm.; biotin, 20 mg.; folic acid, 90 mg.; vitamin B_{12}, 1.35 mg. (Nutritional Biochemicals Corporation, vitamin diet fortification mixture).

[b] Trace mineral mix: KI, 0.264%; $CuSO_4 \cdot 5H_2O$, 0.099%; $ZnSO_4 \cdot 7H_2O$, 0.1749%; $CoCl_2 \cdot 6H_2O$, 0.0165%; NaCl, 55.11%; $MgSO_4 \cdot 7H_2O$, 33.66%; $FeC_6H_5O_7 \cdot 5H_2O$, 9.075%; $Mn(C_2H_3O_2)_2 \cdot 4H_2O$, 1.68%.

[c] Reagent grade minerals were used.

[d] $CaHPO_4 \cdot 2H_2O$, N.F. grade.

and potassium contents of three diets were held constant by variations in the amounts of disodium phosphate, dipotassium phosphate and potassium chloride. Deionized water was available ad libitum. Phosphorus was determined by the A.O.A.C. (1960) method and calcium by atomic absorption spectroscopy (Perkin-Elmer, 1964).

After 8 days on experiment, eight rats from each treatment were placed in metabolism cages for 3 days. Feed consumption was measured and feces and urine collected during this period. Calcium in the urine and in the acid soluble portion of the ashed feces was determined by atomic absorption spectroscopy. At the termination of the experiment (20 days) the rats were killed and the femurs removed for determination of bone ash. Bone ash was determined by the A.O.A.C. (1960) method. All of the results were analyzed for statistical significance by the procedure for paired comparison "t" test (Steel and Torrie, 1960).

Experiment 4. An additional experiment was conducted to determine the relative availability of phosphorus in dicalcium phosphate and disodium phosphate. Three rats from each of 15 litters (total 45 rats) were assigned to three treatment groups as in experiment 3. The low-phosphorus basal diet (table 5) used in this experiment was shown by chemical analysis to contain 0.010% phosphorus and 0.51% calcium. The rats in treatment 1 received no added phosphorus, while rats in treatments 2 and 3 received 0.19% supplemental phosphorus from dicalcium phosphate and disodium phosphate, respectively. Calcium levels in the three diets were maintained constant through

variations in the levels of calcium carbonate. Deionized water was given ad libitum. In accordance with the procedure followed in experiment 3, eight rats from each treatment were placed in metabolism cages for 3 days after having been on the experimental treatments for 8 days. Feed consumption was measured and urine collected during this period. However, due to some diarrhea the feces were not collected. Urine phosphorus was determined by the method of Fiske and SubbaRow (Hawk et al., 1954). After 20 days the rats were killed and the femurs removed. Bone ash determinations were made on the femurs using the method described in experiment 3. Statistical analysis, as in experiment 3, was performed according to the procedure for paired comparison "t" test.

Experiment 5. Since collection of feces was not feasible in experiment 4, the experiment was repeated using 15 non litter mate, male rats per treatment. The same diets were fed in experiments 4 and 5 except 3% of the cerelose was replaced with cellulose (solka-floc) in experiment 5 to control diarrhea. While treatments 1, 2 and 3 were administered as in experiment 4, 15 additional pairs of rats were concurrently pair-fed. One member from each pair was fed the diet containing dicalcium phosphate (treatment P-2) and the other member of each pair was fed the diet containing disodium phosphate (treatment P-3).

After 7 days on experiment, the pair-fed rats were placed in metabolism cages for 72 hours and the feces and urine collected. A second 72-hour collection was made after 18 days on experiment. Urine

phosphorus was determined by the method of Fiske and SubbaRow (Hawk et al., 1954), and fecal phosphorus was determined by a modification of this method on a sample prepared as described for plants by Chapman and Pratt (1961).

Feed consumption for rats not pair-fed (treatments 1, 2 and 3) was determined during a 4-day period in the second week of the experiment and again during the last 3 days of the experiment. No fecal or urine samples were collected from these rats. At the termination of the experiment (21 days), all rats were killed and the femurs removed for the determination of bone ash. Bone ash determinations and statistical analyses were performed as described for the previous experiment.

Results

Experiment 3. Results of this experiment are presented in table 6. The rats receiving treatment 1 (no supplemental calcium) had significantly ($P<.01$) lower average weight gain and bone ash values than those receiving either source of supplemental calcium, and several rats exhibited a negative calcium retention. However, feed consumption did not appear to be affected by the low calcium content of the diet.

There was no significant difference between rats receiving supplemental calcium from dicalcium phosphate or calcium carbonate regarding weight gain, bone ash and feed consumption. Rats receiving dicalcium phosphate excreted significantly ($P<.05$) more calcium in the urine than those receiving calcium carbonate, but there appeared

TABLE 6

CALCIUM AVAILABILITY FROM $CaCO_3$ AND $CaHPO_4 \cdot 2H_2O$ (EXPERIMENT 3)

Treatment number	1	2	3
Calcium supplement	None	$CaHPO_4 \cdot 2H_2O$	$CaCO_3$
Calcium, %[a]	0.015	0.21	0.21
Phosphorus, %[a]	0.39	0.39	0.39
Number of rats	15	15	15
Total gain, gm.[b]	116[c]	138	141
Feed consumption, gm./day[d]	13.0	13.3	13.0
Bone ash, %	31.9[c]	47.2	46.6
Calcium retention, %[d]	----	72.7	81.2
Urine calcium, mg.[d]	----	15.8[e]	8.0
Fecal calcium, mg.[d]	----	7.8	9.4

[a] Air-dry basis.

[b] Period of 20 days.

[c] Differs significantly ($P < .01$) from other treatments.

[d] Average for 8 rats over a 3-day period.

[e] Differs significantly ($P < .05$) from treatment 3.

to be no difference in fecal excretion of calcium. Although rats receiving calcium carbonate had nearly 10% greater calcium retention than rats fed dicalcium phosphate, large variability was present and the difference was not statistically significant.

Experiment 4. The results of this experiment are presented in table 7. The rats that received no supplemental phosphorus (treatment 1) consumed less feed and had smaller weight gain and lower bone ash values than rats fed either source of supplemental phosphorus (significant, $P<.01$). The rats receiving disodium phosphate (treatment 3) gained faster and had a greater percent bone ash than the rats in treatment 2 receiving dicalcium phosphate (significant, $P<.01$). The rats in all treatment groups had some diarrhea and it was not possible to collect feces or calculate phosphorus retention.

Experiment 5. The results of this experiment are presented in table 8. The inclusion of 3% cellulose in the diet appeared to prevent the occurrence of diarrhea. Rats receiving no supplemental phosphorus (treatment 1) consumed less feed, made smaller weight gains and had a lower average percent bone ash than rats receiving supplemental phosphorus from either dicalcium phosphate or disodium phosphate (significant, $P<.01$). The rats receiving disodium phosphate (treatment 3) had greater weight gains than rats receiving dicalcium phosphate (treatment 2), but the difference was not significant. This may be explained by the fact that three rats in treatment 3 consumed 6.3 gm. less feed per day than other rats in this treatment, and had an average weight gain of 72 gm. less. These

TABLE 7
PHOSPHORUS AVAILABILITY FROM $CaHPO_4 \cdot 2H_2O$ AND Na_2HPO_4 (EXPERIMENT 4)

Treatment number	1	2	3
Phosphorus supplement	None	$CaHPO_4 \cdot 2H_2O$	Na_2HPO_4
Phosphorus, %[a]	0.01	0.20	0.20
Calcium, %[a]	0.51	0.51	0.51
Number of rats	15	15	15
Total gain, gm.[b]	17.4[c]	65.8[d]	100.6[e]
Feed consumption, gm./day	8.0[c]	10.2[d]	13.4[e]
Bone ash, %	35.25[c]	42.77[d]	44.80[e]
Urine phosphorus, mg.[f]	----	1.08	0.80

[a] Air-dry basis.

[b] Period of 20 days.

[c,d,e] Treatment means on the same line bearing different superscript letters are significantly ($P < .01$) different.

[f] Period of 72 hours.

TABLE 8

PHOSPHORUS AVAILABILITY FROM $CaHPO_4 \cdot 2H_2O$ AND Na_2HPO_4 (EXPERIMENT 5)

Treatment number	1	2	3	P-2	P-3
Phosphorus supplement	None	$CaHPO_4$[a]	Na_2HPO_4	$CaHPO_4$[a]	Na_2HPO_4
Phosphorus, %[b]	0.01	0.20	0.20	0.20	0.20
Calcium, %[b]	0.49	0.49	0.49	0.49	0.49
Number of rats[c]	15	15	15	14	14
Total gain, gm.[d]	35.8[d]	82.1	92.9	36.3	44.9
Feed consumption, gm./day	9.1[e]	12.3	13.3	----	----
Bone ash, %	21.49[d]	39.07	38.24	39.94[f]	42.62
Phosphorus retention, %[g]	----	----	----	60.3[f]	83.9
Urine phosphorus, mg.[g]	----	----	----	3.3	3.1
Urine phosphorus, mg.[g]	----	----	----	8.5	8.9
Fecal phosphorus, mg.[g]	----	----	----	278[f]	131

[a] $CaHPO_4 \cdot 2H_2O$.

[b] Air-dry basis.

[c] Originally 15 rats in each treatment, but 1 rat died in treatment P-2 early in the experiment and the rat from that pair in treatment P-3 was removed at this time.

[d] Period of 21 days.

[e] Differs significantly ($P < .01$) from treatments 2 and 3.

[f] Differs significantly ($P < .01$) from treatment P-3.

[g] Average of two 72-hour periods.

rats also had a low bone ash (26.5 to 31.0%) resulting in an average percent bone ash for the group slightly lower than for rats receiving dicalcium phosphate.

The pair-fed rats receiving disodium phosphate, treatment P-3, gained faster and had a greater percent bone ash than those receiving dicalcium phosphate, treatment P-2 (significant, $P<.01$). In addition, the rats in treatment P-3 retained approximately one-third more phosphorus (significant, $P<.01$). There appeared to be no difference in urinary phosphorus excretion, but fecal phosphorus excretion was higher in rats receiving dicalcium phosphate (significant, $P<.01$).

Discussion

On the basis of weight gain, bone ash and feed consumption there was no difference in the availability of calcium from dicalcium phosphate or calcium carbonate. This is in agreement with earlier work with cattle (Lindsey and Archibald, 1925; Turner et al., 1927; Haydern et al., 1930; Lantow 1933; Hansard, et al., 1957), swine (Combs and Wallace, 1962) and poultry (Dilworth et al., 1964) showing that calcium from a variety of calcium supplements, soft phosphates excluded, and natural feeds appear to be equally available.

Although calcium availability from the supplements used in the experiments reported herein did not appear to differ, the rats receiving dicalcium phosphate excreted higher levels of calcium in the urine than those receiving calcium carbonate. The increased urinary calcium excretion may account for the slightly lower (nonsignificant)

calcium retention observed in rats fed dicalcium phosphate. However, because of large differences known to exist between ruminants and monogastric animals with regard to urinary calcium excretion, these data may not be directly applicable to sheep.

The relatively high dietary value of the calcium phosphate, including dicalcium phosphate, as sources of phosphorus for monogastric animals and ruminants is well known. However, results obtained in experiments 4 and 5 indicate that phosphorus from disodium phosphate is even more available than phosphorus from dicalcium phosphate. Data obtained with the pair-fed rats indicate that phosphorus retention in rats fed disodium phosphate was 39% greater than phosphorus retained by rats fed dicalcium phosphate. Since the amount of phosphorus added to the diet was less than that which is required for optimum growth, average urinary phosphorus excretion was relatively low in both groups. Although these data were obtained with rats, it appears that the higher incidence of phosphatic urinary calculi in ruminants associated with disodium phosphate when this compound and dicalcium phosphate are fed at equivalent levels of calcium and phosphorus is probably related to the high degree of phosphorus availablity associated with disodium phosphate.

Increased levels of dietary phosphorus (Bushman et al., 1965a, 1965b; Emerick and Embry, 1963, 1964; Robbins et al., 1965) and high levels of urinary phosphorus (Bushman et al., 1965a, 1965b; Robbins et al., 1965; Crookshank et al., 1965) have previously been shown to be associated with a high incidence of phosphatic urolithiasis in

lambs. However, Packett and Hauschild (1964) found elevated serum and urine phosphorus levels to be associated with urinary calculi formation in lambs fed rations containing normal levels (0.26 and 0.30%) of phosphorus. Further, Crookshank et al. (1965) reported an increase of urolithiasis in lambs fed a pelleted ration, the increase in urolithiasis being accompanied by increases in serum and urine phosphorus values. Unfortunately phosphorus availability was not determined in their study. However, Slinger et al. (1966) have found that pelleting increases the apparent phosphorus availability in poultry rations.

Summary

Male Sprague-Dawley rats were used in 3 experiments to compare the relative availability of calcium in calcium carbonate and dicalcium phosphate and phosphorus in disodium phosphate and dicalcium phosphate. Weight gain, bone ash and net retention values were used as critera for evaluation.

In the first experiment no significant differences in the availability of calcium were obtained between the two sources of calcium. However, rats receiving dicalcium phosphate excreted more calcium in the urine than those receiving calcium carbonate.

In the second and third experiments, phosphorus from disodium phosphate appeared to be approximately one-third more available than phosphorus from dicalcium phosphate. The data interpreted in the light of previous experiments with sheep suggest that differences in phosphorus availability may account for the differences in urinary

calculi incidence associated with the feeding of rations containing dicalcium phosphate or disodium phosphate and calcium carbonate at equivalent levels of calcium and phosphorus.

EXPERIMENTS 6 AND 7. EFFICACY OF VARIOUS CHLORIDES AND CALCIUM CARBONATE IN THE PREVENTION OF URINARY CALCULI

Several dietary supplements have been shown to reduce the incidence of phosphatic urinary calculi in livestock. Most prevalent among these have been calcium carbonate, sodium chloride and ammonium chloride.

Udall and Chow (1963) postulated that the effect of sodium chloride in preventing urinary calculi was exerted through an increase in urinary excretion of the chloride ion and subsequent competition for cationic binding sites on matrix materials. On the basis of this and the apparent beneficial effects obtained from increased levels of dietary calcium, it was postulated that calcium chloride might provide a high degree of protection against urinary calculi. Therefore, these experiments were conducted to compare calcium chloride, ammonium chloride, sodium chloride and calcium carbonate as preventatives of urinary calculi in sheep fed rations previously shown to be calculogenic, and to determine the effect of ammonium chloride on the palatability of protein supplements.

Experimental

Experiment 6. This experiment, conducted over an 84-day period during summer and early fall, utilized 234 wether lambs of predominantly Hampshire breeding. The lambs, averaging approximately 29 kg., were allotted to 9 replicated treatments on the basis of weight with 13 lambs per lot initially.

The lambs were fed a basal ration consisting of the following in

percent: ground shelled corn, 74.7; ground alfalfa hay, 20; soybean meal, 3.2; dibasic sodium phosphate, 1.6; and trace mineralized salt, 0.5. This ration was formulated to contain 11% crude protein (calculated), and was shown by chemical analysis to contain 0.62% phosphorus, 0.37% calcium, 0.19% magnesium and 0.30% chloride ion. Rations containing similar levels of phosphorus and calcium have previously been shown at this station to promote a high incidence of urinary calculi in sheep (Emerick and Embry, 1963, 1964; Bushman et al., 1965a, 1965b).

Treatments in addition to the control consisted of ammonium chloride, calcium chloride, sodium chloride and calcium carbonate, each fed at levels of 0.5 and 1.5% of the ration. Commercial grade, nonhydrated chloride salts and feed grade calcium carbonate (ground limestone) were used. The rations were full-fed once daily and water was available at all times.

A blood sample was obtained by jugular vein puncture from each lamb after 25 days on experiment, and the serum was stored frozen until analyzed. Overnight (16 hr.) urine collections were obtained from one lamb per treatment daily, alternating replications, until a sample had been obtained from 16 lambs on each treatment. Urine collections were made by use of attached rubber urinals which allowed free movement of the animals. Serum and urine calcium and magnesium were determined by atomic absorption spectroscopy (Perkin-Elmer, 1964), serum and urine phosphorus by the method of Fiske and SubbaRow (Hawk et al., 1954) and urine chloride by the method of Volhar-Arnold (Hawk et al.,

1954). In the analyses of the basal ration, calcium and magnesium were determined on the acid soluble portion of the feed ash by atomic absorption spectroscopy; phosphorus and chloride were determined by A.O.A.C. (1960) method.

The lambs were observed for symptoms of urinary calculi throughout the experiment. Animals with apparent blockage of the urinary tract were slaughtered, and the urinary tracts were examined. At the termination of the experiment, the remaining lambs were slaughtered and the urinary bladders and kidneys were examined for calculi.

Data pertaining to average daily gain and feed consumption were calculated only for those lambs finishing the experiment. Since lambs with urinary calculi were removed from the experiment when symptoms were first observed, feed consumption data were corrected by subtracting an average value for each lamb removed.

A statistical analysis of urinary calculi incidence among treatments was made by the chi-square method, and weight gain and carcass grade by the method of least squares. Statistical analysis of serum calcium, phosphorus and magnesium was made by the method of least squares, and comparisons of treatment means against the control means were performed according to Dunnett's procedure (Steel and Torrie, 1960). Due to the large differences in urine volumes both within and between treatments, the urinary calcium, phosphorus and magnesium values were adjusted by covariance analysis, and comparisons of treatment means against the control means were performed according to Dunnett's procedure.

Experiment 7. An additional experiment was conducted to determine the palatability of ammonium chloride when fed in corn or corn-soybean meal supplements which were only partially mixed with the remainder of the ration. Treatments involved supplements containing two (0 and 75%) levels of soybean meal and three levels (0, 8 and 16%) of ammonium chloride in a 2 x 3 factoral design. The 75% soybean meal and 16% ammonium chloride provided similar levels of nitrogen. Each treatment was replicated with 11 ewe lambs per group (total 132 lambs). The lambs were predominately of Hampshire breeding and averaged 39 kg. initally. Allottment was on the basis of weight.

The rations used at the beginning of this experiment (period 1) consisted of 90% ground ear corn and 10% supplement. Dicalcium phosphate, trace-mineralized salt and water were available ad libitum. At the time of feeding, the treatment supplements were placed in the feeders on top of the remainder of the ration and mixed lightly by hand. Under these conditions, supplements containing ammonium chloride were not readily consumed. After six weeks, the ear-corn portion of the rations was changed to equal parts of ground shelled corn and corn silage (wet-weight basis). This change was administered during the second 6-week period (period 2). During this time, the supplements comprising the treatments were fed at a constant rate of 136 gm. per lamb daily. This level resulted in a daily intake of 9.5 and 19 gm. ammonium chloride daily with supplements containing 8% and 16% of the compound.

Feed analyses were performed according to the procedures outlined

for experiment 6. The rations, without consideration for the additional minerals fed ad libitum, contained an average of 0.31% phosphorus and 0.04% calcium in period 1, and 0.20% phosphorus and 0.07% calcium in period 2. At the termination of the experiment, all lambs were slaughtered, and the urinary bladders and kidneys were examined for calculi.

Results

Experiment 6. Data from this experiment are shown in table 9. No significant differences between treatments were observed for rate of gain or carcass grade. A low rate of gain for all groups initially was believed to have resulted from a heavy internal parasite infestation existing when the lambs were placed on experiment. The lambs were treated after about 4 weeks with thiabendazole and lead arsenate. The gains improved following these treatments with all groups gaining in excess of 0.18 kg. per day, with the exception of the control group which continued to gain at a lower rate. The average carcass grade for each group of lambs was choice.

Since group feeding was employed, no statistical analysis was applied to feed consumption or feed efficiency. However, all rations were consumed readily, and none of the treatments used in this experiment adversely affected feed consumption.

A 50% incidence of urinary calculi occurred in the control lambs. Treatments providing 1.5% calcium chloride or ammonium chloride resulted in significantly ($P<.01$) lower incidences urinary calculi. No clinical cases were noted with either treatment, and only one lamb

TABLE 9. EFFECTS OF FEEDING AMMONIUM CHLORIDE, CALCIUM CHLORIDE, SODIUM CHLORIDE AND CALCIUM CARBONATE IN COMPLETE RATIONS FOR SHEEP

Supplement[a]	Control	NH_4Cl		$CaCl_2$		NaCl		$CaCO_3$	
		0.5%	1.5%	0.5%	1.5%	0.5%	1.5%	0.5%	1.5%
Number of lambs[b]	24	24	24	24	22	21	24	25	25
Av. daily gain, kg.	0.111	0.139	0.153	0.133	0.170	0.149	0.136	0.139	0.150
Daily ration, kg.[c]	1.09	1.16	1.17	1.19	1.26	1.16	1.28	1.21	1.24
Feed/kg. gain, kg.[c]	9.41	8.83	7.58	8.81	7.38	8.28	10.33	8.76	8.40
Carcass grade[d]	11.9	11.6	11.6	12.5	12.0	11.3	11.6	11.6	11.6
Serum values, mg. per 100 ml.									
Calcium	9.38	10.03	9.46	9.71	10.32**	9.57	9.19	10.15**	10.30**
Phosphorus	9.86	8.65	8.87	8.56	8.77	9.24	9.94	8.44	9.83
Magnesium	2.73	2.37**	2.33**	2.58	2.50	2.65	2.49	2.41	2.52
Urine values, mg. per 100 ml.[e]									
Calcium	5.51	7.46	22.93**	6.14	14.20**	3.78	6.03	6.25	4.78
Phosphorus	21.95	8.53	31.87	13.70	12.68	18.80	12.03	21.80	10.60
Magnesium	152	118	117	134	117	92	92	115	101
Chloride	347	623*	919*	620*	996*	596	825*	352	340
Urine volume, ml.	408	447	527	526	447	777	750	466	638
Urine pH	8.24	8.35	6.43**	8.31	7.85*	8.45	8.16	8.44	8.55
Urinary calculi incidence									
Clinical[f]	6	9	0	9	0	6	7	6	3
Total[g]	12	10	1**	11	1*	8	8	11	6

[a] Air-dry basis.
[b] Initially 25 lambs per treatment, but some losses occurred due to causes unrelated to the experiment.
[c] Calculated only for the animals surviving the entire experimental period.
[d] Choice = 11, Choice + = 12, Prime - = 13.
[e] Data from 16 lambs per treatment.
[f] Death due to urine blockage or slaughtered when death appeared certain.
[g] Includes losses due to urine blockage and animals having urinary mineral deposits at termination of the experiment.
* Different ($P < .05$) from control mean.
** Different ($P < .01$) from control mean.

in each of these two treatments had urinary calculi when slaughtered. A lower level (0.5%) of calcium chloride or ammonium chloride appeared to be ineffective in calculi prevention. While there was a trend toward a lower incidence of calculi in lambs fed 0.5 or 1.5% of sodium chloride (38 and 33% incidence, respectively) and those fed the higher level of calcium carbonate (24% incidence), the values did not differ significantly from that obtained in the controls.

Serum calcium values were increased in lambs fed either level of calcium carbonate (significant, $P<.01$), the higher level of calcium chloride ($P<.01$) or the lower level of ammonium chloride ($P<.05$). Urinary calcium concentration was increased ($P<.01$) in lambs fed the highest level of ammonium chloride or calcium chloride. These two treatments also significantly ($P<.05$) lowered urinary pH, but the average value was in the acidic range only in the lambs fed 1.5% ammonium chloride. Serum and urine phosphorus was not significantly affected by the various treatments.

When compared with the controls, average serum magnesium values tended to be lower in all treatment groups. This difference reached significance ($P<.01$) for lambs fed either level of ammonium chloride or the lower level of calcium carbonate. However, urine magnesium was not affected.

Urinary chloride concentration was increased significantly ($P<.05$) in all groups of lambs receiving a supplemental source of dietary chloride except those fed the low level (0.5%) of sodium chloride. In the latter group, the increase approached significance

at the 5% level of probability. The magnitude of urinary chloride concentrations appeared related to the level fed, but there was no significant difference between sources, i.e., ammonium chloride, calcium chloride or sodium chloride.

Experiment 7. Results of this experiment are presented in table 10. During period 1 (6 weeks) feed consumption and weight gain were reduced when supplements contained either 8% or 16% ammonium chloride. The effect was less detrimental when ammonium chloride was fed in conjunction with soybean meal. Lambs receiving the highest level of ammonium chloride lost weight when soybean meal was not included and gained only 0.012 kg. per day with soybean meal in the supplement.

When the rations were changed to ground corn and corn silage (period 2), feed consumption and average daily gain of all lambs were improved. With this ration, feed consumption and weight gain were not affected by the 8% ammonium chloride supplements but were lowered by those containing 16%. With supplements containing 16% ammonium chloride, soybean meal greatly improved feed consumption and weight gain but the values were still below those obtained for the controls.

The occurrence of urinary calculi was restricted to lambs in groups receiving less than 19 gm. (16% in the supplement) of ammonium chloride daily. Lambs receiving the 8% ammonium chloride supplement had a 7% incidence of urinary calculi and the controls had an incidence of 11%. The difference in the incidence (0 vs. 7%) of calculi between the two levels of ammonium chloride approached significance at the 5% level of probability.

TABLE 10. EFFECT OF AMMONIUM CHLORIDE IN A SUPPLEMENT FOR SHEEP

Supplement number[a]	1	2	3	4	5	6
Ammonium chloride, %[b]	0.0	8.0	16.0	0.0	8.0	16.0
Soybean oil meal, %[b]	0.0	0.0	0.0	75.0	75.0	75.0
Ground corn, %[b]	100.0	92.0	84.0	25.0	17.0	9.0
Number of lambs[c]	22	22	22	22	22	21
Average daily gain, kg.						
period 1	0.114	0.029	-0.038	0.143	0.098	0.012
period 2	0.150	0.168	0.039	0.175	0.174	0.136
Daily ration, kg.						
period 1	1.04	0.79	0.61	1.09	0.98	0.75
period 2						
concentrate	0.82	0.77	0.33	0.87	0.84	0.64
silage	0.65	0.75	0.43	0.69	0.66	0.62
Urinary calculi incidence[d]	3	1	0	2	2	0

[a] Supplements were fed as 10% of air-dry rations (period 1), or 136 gm. per lamb daily (period 2).

[b] Air-dry basis.

[c] Initially 22 lambs per treatment but one loss occurred in group 6 by a cause which was unrelated to the experiment.

[d] Incidence found at slaughter.

Discussion

Ammonium chloride and calcium chloride fed at levels of 1.5% of a calculogenic ration were found to be equally effective in the prevention of urinary calculi. Each of these compounds was more effective than comparable levels of either calcium carbonate or sodium chloride, even though they provided similar amounts of calcium and/or chloride. Lower levels (0.5%) of these compounds was ineffective.

Leoschke and Elvehjem (1954)used 1 gm. of ammonium chloride per animal daily to prevent phosphatic urinary calculi formation in mink. They attributed its protective action to an acidification of the urine, and effect observed in the studies reported herein. Crookshank et al. (1960) reported a reduction in phosphatic urinary calculi formation in steers receiving 90 gm. ammonium chloride daily. However, the use of calcium chloride for the prevention of phosphatic urinary calculi has not been previously documented.

Data reported previously (Bushman et al., 1965b) concerning the use of calcium carbonate for the prevention of phosphatic ovine urinary calculi indicated that the dietary calcium-to-phosphorus ratio should be at least 2:1. The ration containing 1.5% of calcium carbonate used in the current studies provided a calcium-to-phosphorus ratio approximating 1.5:1 and gave only partial protection against urinary calculi.

Emerick and co-workers (Emerick et al., 1959; Bushman et al., 1965a, 1965b) and Packett and Hauschild (1964) have previously presented data indicating that an elevated urinary phosphorus level is

probably the most important measurable factor predisposing the development of phosphatic urinary calculi in lambs. However, no clear relationship between urinary phosphorus levels and the incidence of urinary calculi was seen in the current studies. A tendency toward higher urinary phosphorus levels in the lambs fed 1.5% of ammonium chloride while remaining essentially free of urinary calculi indicates that the relationship between urinary phosphate and phosphatic calculi is not upheld under conditions resulting in an acidic urine. This observation is supported by the demonstration of Crookshank et al. (1960) that dietary phosphoric acid acts to reduce calculi formation.

Sheep receiving either of the two levels of sodium chloride exhibited a tendency toward higher urine volumes than those on all other treatments, and urinary chloride concentrations that were only slightly lower than those found for sheep receiving comparable levels of ammonium chloride or calcium chloride. The lack of a significant protective effect attributable to sodium chloride under these conditions fails to support the postulation of Udall and Chow (1963) that an increase in urinary chloride excretion protects against calculi formation through ion competition. However, the levels of sodium chloride used in these studies were lower than the 4% (Udall, 1959, 1962) or 10% (Elam et al., 1957) levels reported previously to give a significant reduction of urinary calculi in sheep.

Both ammonium chloride and calcium chloride when fed at the highest levels (1.5%) increased urinary calcium concentration and lowered urine pH. Gill et al. (1959) have previously reported that the

urinary excretion of calcium in rats increased when the urine was made acidic by feeding ammonium chloride. However, it has previously been reported that the reduction of urinary calculi in sheep by the feeding of calcium carbonate did not appear to involve an increase in urinary calcium (Schneider et al., 1952; Bushman et al., 1965a), but appeared to be related to a decrease in urine phosphorus. Gill et al. (1959) reported that the protective effect of calcium lactate against phosphatic urolithiasis in rats appeared to involve a decreased intestinal absorption of phosphorus and a subsequent reduction in urinary phosphorus excretion. Thus, there appears to be no basis for assigning a role in the prevention of urinary calculi to the increased urinary calcium concentration in the presence of an acidified urine.

Udall and co-workers (Udall, 1962; Udall and Chow, 1963) reported that a reduction in urine pH did not appear to affect stone formation, and that calculi occurred in either acidic or alkaline urine. On the other hand, Leoschke and Elvehjem (1954) and Crookshank et al. (1960) have indicated that alkaline urine appears more conducive to phosphatic urolithiasis than acidic urine. These authors obtained a reduction in urine pH and a subsequent reduction in calculi formation by feeding ammonium chloride to mink and cattle, respectively. They attributed the protective effect of the ammonium chloride to a reduction in urinary pH.

The compounds used in experiment 6 were thoroughly mixed into a complete ration using a mechanical mixer. Under these conditions, no adverse effects of the treatments were noted concerning feed

consumption or weight gain. In experiment 7, ammonium chloride was mixed into a supplement comprising 10% of the total ration, the supplement being incompletely mixed with the remainder of the ration by hand at the time of feeding. Inadequate mixing of the ammonium chloride tended to make the ration unpalatable, resulting in lower feed consumption and weight gains where levels of the compound were adequate to prevent urinary calculi. Including soybean meal in the supplement was partially effective in increasing the palatability of the rations containing ammonium chloride. Lower weight gain was associated with the feeding of ammonium chloride, even though the supplement containing the highest level (16%) of this compound provided an amount of crude protein equivalent to that provided by the 75% soybean meal supplement. In no instance did lambs receiving ammonium chloride in addition to soybean meal make greater weight gains than were made by lambs receiving soybean meal alone.

The data show that either ammonium chloride or calcium chloride can be used successfully to prevent urinary calculi, however, high levels of ammonium chloride may be unpalatable.

Summary

Wether lambs averaging 20 kg. were used in nine treatment groups of 21 to 25 lambs each and fed a known calculogenic basal ration to which either ammonium chloride, calcium chloride, sodium chloride or calcium carbonate was added. Each compound was fed as 0.5 and 1.5% of the diet.

Feeding either 1.5% ammonium chloride or calcium chloride

resulted in a significant reduction in urolithiasis. The incidence was 4, 4, 33 and 24%, respectively, when ammonium chloride, calcium chloride, sodium chloride and calcium carbonate were fed at the 1.5% level, and 42, 46, 38 and 44%, respectively, when fed at the 0.5% level. The controls had a 50% incidence of calculi.

Feeding ammonium chloride or calcium chloride at the 1.5% level also significantly lowered urine pH and significantly increased urinary calcium excretion. None of the compounds had a detrimental effect upon feed consumption, rate of gain or carcass grade when fed at either level.

In a second experiment, 132 ewe lambs averaging 39 kg. were fed supplements containing two levels of soybean meal (0 and 75%) and three levels of ammonium chloride (0, 8 and 16%) in a 2 x 3 factorially designed experiment. The supplements, comprising 10% of the total ration, were fed as a top-dressing and were only partially mixed with the remainder of the ration. When fed with a ground ear corn ration (period 1), both levels of added ammonium chloride lowered feed consumption. With a change of the ration to include corn silage (period 2), 8% ammonium chloride in the supplement was neither detrimental nor benificial, but 16% ammonium chloride reduced feed consumption and weight gain. Feeding the highest level of ammonium chloride (16% in the supplement), resulting in an intake of approximately 19 gm. per head daily, significantly decreased the incidence of urinary calculi.

EXPERIMENT 8. EFFECT OF VARIOUS CHLORIDES AND CALCIUM CARBONATE ON CALCIUM, PHOSPHORUS, SODIUM, POTASSIUM AND CHLORIDE EXCRETION, AND RETENTION, AND THEIR RELATIONSHIP TO URINARY CALCULI IN LAMBS

This experiment was conducted: (1) to further determine the degree of protection afforded sheep against urolithiasis by the feeding of various salts, and (2) to determine the effect of the salts on excretion and retention of various ions. In addition to the salts used in experiment 6, potassium chloride was included in this study. A protective effect against phosphatic urolithiasis has been assigned to dietary potassium by Robbins et al. (1965), and Crookshank (1966) has presented data indicating a greater protective effect for various potassium salts compared with corresponding sodium salts. However, Elam et al. (1956) reported that feeding potassium carbonate increased the incidence of calculi in lambs.

Experimental

This study consisted of a feeding trial and balance trials. In the feeding trial, data pertaining to feedlot performance and urinary calculi incidence were obtained during an 88-day period under conditions of ad libitum feeding. In the balance trials, excretion and balance data were obtained during 7-day collection periods under conditions of equal and constant feed intake.

Feeding trial. One hundred twenty crossbred wether lambs weighing approximately 38 kg. were allotted on the basis of weight into six replicated treatments. They were fed a basal ration consisting of ground shelled corn, 75.0%; ground alfalfa hay, 20.0%; soybean

oil meal, 2.9%; disodium phosphate, 1.6%; and trace mineralized salt, 0.5%. This ration contained 11% crude protein (calculated) and was shown by chemical analysis to contain 0.64% phosphorus, 0.33% calcium, 0.30% chloride, 0.87% sodium and 0.67% potassium. Similar rations have previously been used at this station for the experimental production of phosphatic urinary calculi (Bushman et al., 1965b).

Treatments in addition to the control consisted of the following additions to the basal ration: ammonium chloride, 1%; calcium chloride, 1%; potassium chloride, 1%; sodium chloride, 4%; and calcium carbonate, 2%. Commerical-grade anhydrous chloride salts and feed-grade calcium carbonate (ground limestone) were used. The rations were fed once daily in an amount so feed would be available at all times, and water was offered ad libitum.

The feed samples were ashed according to the procedure of Chapman and Pratt (1961) for plants, and the analysis for calcium, phosphorus, sodium and potassium were performed on the acid-soluble ash. Calcium was determined by atomic absorption spectroscopy (Perkin-Elmer, 1964), and phosphorus by a modification of Fiske and SubbaRow's procedure for urine (Hawk et al., 1954). Sodium and potassium were determined by flame photometry correcting for sodium interference in the potassium analysis. The soluble chloride was determined by the A.O.A.C. (1960) procedure.

The lambs were observed for symptoms of urinary calculi throughout the experiment. Lambs with apparent blockage were slaughtered and the urinary tracts examined. At the termination of the

experiment, the remaining lambs were slaughtered and the urinary bladders and kidneys examined for calculi. Data pertaining to average daily gain and feed consumption were calculated only for those lambs finishing the experiment. Since lambs with urinary calculi were removed from the experiment when symptoms were first observed, feed consumption data were corrected by subtracting an average value for each lamb removed.

A statistical analysis of urinary calculi incidence among treatment groups was made by the chi-square method (Steel and Torrie, 1960). Statistical analysis of weight gain data was made by the method of least squares, and comparison of treatment means against the control mean was performed according to Dunnet's procedure.

Balance trials. An additional 96 wether lambs were fed the basal ration used in the feeding trial, omitting disodium phosphate during the pretreatment period. At 3-week intervals, 24 lambs were removed, allotted into six treatments of four lambs each and placed in metabolism cages. This procedure was repeated four times with different lambs being used each time. The treatments during these periods were identical to those used in the feeding trial.

After being placed in the metabolism cages, the lambs were allowed 2 weeks to adapt to the treatments. Following the adaptation period, urine and feces were collected twice daily for a period of 7 days. The lambs were fed 454 gm. of the appropriate ration twice daily during the adaptation and collection periods. Water was available at all times and water consumption was measured during the

collection periods. Lambs failing to eat during the latter part of the adaptation period or during the collection period were removed from the experiment.

Urine pH was determined at each collection and a 10% aliquot of urine was saved and pooled over the 7-day collection period. The urine samples were preserved with 2% of concentrated sulfuric acid (v/v), and the feces were dried prior to storage. At the end of the collection period, a blood sample was obtained by jugular vein puncture and the serum stored frozen.

The feces samples were analyzed by the methods described for feed analysis in the feeding trial. For serum and urine samples, calcium was determined by atomic absorption spectroscopy (Perkin-Elmer, 1964), phosphorus was determined by the method of Fiske and SubbaRow (Hawk et al., 1954), and sodium and potassium were determined by flame photometry (Coleman, 1956). Serum chloride was determined by direct titration using the method of Schales and Schales (Hawk et al., 1954), and urine chloride was determined by the method of Volhard-Arnold (Hawk et al., 1954). Drinking water was analyzed for calcium by the permanganate method (A.P.H.A., 1955), sodium and potassium by flame photometry and chloride by mercuric nitrate titration (A.P.H.A., 1955). The water contained 94, 10, 16 and 312 ppm of calcium, chloride, sodium and potassium, respectively. The phosphorus content of this source of water is known to be negligible.

Statistical analysis of each of the ions determined in the serum, urine and feces, as well as net retention, urine volume and pH was

performed by the method of least squares. Comparisons of treatment means against the control means were performed according to Dunnet's procedure (Steel and Torrie, 1960).

Results and Discussion

Feeding trial. Data pertaining to the feedlot trial are presented in table 11. Control lambs had a 50% incidence of urinary calculi. The feeding of ammonium chloride resulted in a significant ($P < .01$) reduction of urinary calculi with only one nonobstructive case occurring in lambs on this treatment. Lambs receiving calcium chloride had one obstructive case of calculi early in the experiment and two additional cases that were encountered at slaughter. This reduction attributed to calcium chloride, 16% incidence vs. a 50% incidence in the controls, approached significance at the 5% level of probability. Data have been reported previously indicating that ammonium chloride (Leoschke and Elvehjem, 1954; Crookshank et al., 1960; Bushman et al., 1967) and calcium chloride (Bushman et al., 1967) are effective in preventing phosphatic urolithiasis.

In contrast to results reported by Crookshank (1966), feeding 1% potassium chloride significantly ($P < .05$) increased the incidence of urinary calculi, resulting in a total (obstructive plus nonobstructive) incidence of 85%. In this instance obstructive cases alone accounted for a 35% incidence. Elam et al. (1956) earlier reported that the feeding of 1.9% potassium carbonate increased the incidence of urinary calculi in lambs with the greatest effect being obtained when it was fed in conjunction with phosphoric acid.

TABLE 11. EFFECT OF VARIOUS SALTS FED TO SHEEP, PHASE 1

Treatment	Control	1% NH_4Cl	1% $CaCl_2$	1% KCl	4% NaCl	2% $CaCO_3$
Number of lambs[a]	20	20	19	20	20	20
Average daily gain, kg.[b]	0.157	0.126	0.160	0.114*	0.137	0.162
Daily ration, kg.[b]	1.330	1.287	1.403	1.224	1.240	1.364
Feed per kg. gain, kg.[b]	8.45	10.19	8.70	10.74	9.12	8.35
Carcass grade[b,c]	12.42	12.10	12.36	11.87	12.00	12.36
Urinary calculi incidence						
Clinical[d]	3	0	1	7	4	2
Total[e]	10	1**	3	17*	7	6

[a] Originally 20 lambs per treatment, but one lamb died from enterotoxemia.

[b] Calculated only for those lambs surviving the entire experimental period.

[c] Choice = 11, Choice + = 12, Prime - = 13.

[d] Death due to urine blockage, or slaughtered when death appeared certain.

[e] Includes losses due to urine blockage and animals having mineral deposits at termination of the experiment.

* Significantly ($P < .05$) different from the corresponding control mean.

** Significantly ($P < .01$) different from the corresponding control mean.

While feeding 4% sodium chloride or 2% calcium carbonate appeared to lower the incidence of urinary calculi, the reductions were not significant. The feeding of 4% sodium chloride has previously been shown to reduce phosphatic urolithiasis in lambs (Udall, 1962) and siliceous urolithiasis in calves (Bailey, 1967). Data previously reported from this station (Bushman et al., 1965b) showed that the protective effect of calcium carbonate added to high phosphorus rations improved up to the highest calcium-to-phosphorus ratio (2.3:1) used. The calcium-to-phosphorus ratio of the 2% calcium carbonate ration fed in the current experiment was 1.7:1.

Since group feeding was employed, statistical analysis was not performed on feed consumption or feed efficiency data. However, lambs receiving ammonium chloride, potassium chloride or sodium chloride had the lowest feed consumption values and subsequently the lowest average daily weight gains. Average daily gain of lambs fed potassium chloride was significantly ($P < .05$) lower than that attained by control lambs. A reduction in weight gain attributable to ammonium chloride approached significance at the same level of probability. Lambs making the lower weight gains had slightly, but not significantly, lower carcass grades. In previous work (Bushman et al., 1967), ammonium chloride fed at a slightly higher level (1.5%) than the 1% used in this study had no affect on performance of lambs when it was mixed in a complete ration, but an equivalent amount fed in a supplement lowered feed consumption and weight gain.

Balance trials. Data pertaining to the balance trials are

presented in table 12. Feeding either calcium chloride or calcium carbonate resulted in a significant ($P<.01$) increase in calcium retention. Of these two compounds, only calcium chloride resulted in an increase in calcium concentration and excretion in the urine. This increase in excretion was significant ($P<.05$), and the increase in concentration in mg. of calcium per 100 ml. of urine approached significance at this level of probability. Both calcium sources significantly ($P<.01$) increased fecal calcium.

Ammonium chloride significantly ($P<.05$) increased both the concentration and total excretion of urinary calcium. However, the variation was not apparent in the fecal calcium or calcium retention values, and calcium excretion in the urine was relatively small in all instances with a maximum of 0.5 gm. being excreted in 7 days. Potassium chloride and sodium chloride had no effect on excretion or retention of calcium.

Bushman et al. (1967) previously reported that feeding 1.5% of ammonium chloride or calcium chloride increased the concentration of calcium in the urine of lambs. It was suggested at that time that increased urinary calcium was probably not a primary factor in the reduction of urinary calculi. Rather, the mode of action of these two compounds was thought to be a reduction in urinary pH. This is supported by the data herein showing that only ammonium chloride resulted in a significant ($P<.01$) decrease in urinary pH with this being the only treatment yielding a significant reduction in the incidence of calculi.

TABLE 12. BALANCE-TRIAL DATA, PHASE 2.

Treatment	Control	1% NH_4Cl	1% $CaCl_2$	1% KCl	4% NaCl	2% $CaCO_3$
Number of lambs	13	16	14	13	13	12
Retention, gm./7 days						
Calcium	4.14	4.34	11.85**	4.30	4.47	23.66**
Phosphorus	10.02	12.68	10.57	11.43	12.17	11.86
Chloride	4.34	14.38	17.77*	11.39	31.20**	1.50
Sodium	23.64	22.79	21.78	22.60	61.17**	20.62
Potassium	9.84	13.67	9.59	22.38**	10.03	13.04
Urine values mg./100 ml.						
Calcium	1.36	6.84**	4.40	2.02	2.18	1.66
Phosphorus	24.39	39.01	14.88	21.76	20.75	7.69
Chloride	170.1	679.2**	461.9*	491.3*	943.6**	268.4
Sodium	292.1	385.8	316.1	329.3	593.5**	487.0
Potassium	281.9	296.3	284.1	503.8*	173.6	378.4
Urine excretion, gm./7 days						
Calcium	0.127	0.506*	0.503*	0.139	0.354	0.121
Phosphorus	1.925	3.101	1.969	1.672	3.318	0.710
Chloride	14.30	45.46**	40.86**	34.11**	137.00**	17.35
Sodium	24.01	26.03	30.07	23.25	87.06**	30.86*
Potassium	22.87	21.23	27.94	37.90*	25.61	24.56
Fecal excretion, gm./7 days						
Calcium	17.55	17.50	32.72*	16.51	18.36	45.34**
Phosphorus	26.71	23.76	26.07	24.11	23.48	26.49
Sodium	5.353	5.162	2.256**	5.083	5.975	2.727**
Potassium	7.966	6.865	4.322**	7.879	5.713*	3.840**
Urine volume, ml./day	1277	1295	1942	1172	2704*	1122
Urine pH	8.73	8.35**	8.55	8.74	8.51	8.78

[a] Initially 16 lambs per treatment, but some lambs had to be removed for failure to adapt to the metabolism cages.

* Significantly ($P < .05$) different from the corresponding control mean.

** Significantly ($P < .01$) different from the corresponding control mean.

The reduction of urinary pH attributed to the feeding of 1% ammonium chloride in this study was considerably smaller than that obtained by feeding 1.5% of ammonium chloride in the previous experiment (Bushman et al., 1967). A level of 0.5% ammonium chloride had no effect on urinary pH in the previous experiment.

There was no significant affect on phosphorus retention or excretion by any of the compounds fed. However, there was a trend toward increased urinary phosphorus excretion in lambs fed 1% ammonium chloride, and the feeding of calcium carbonate resulted in a reduction in urinary phosphorus excretion approaching significance at the 5% level of probability. Lueker and Lofgreen (1961) have demonstrated that variations in dietary calcium levels inversely affect phosphorus absorption and urinary phosphorus excretion in sheep. This has been concluded by Gill et al. (1959) to be the principal mechanism whereby calcium carbonate reduces the incidence of urinary calculi.

The retention of chloride was significantly ($P < .05$) increased by feeding either calcium chloride or sodium chloride. While the feeding of ammonium chloride or potassium chloride appeared to increase retention of the chloride ion, the differences were not significant.

All of the chloride supplements significantly increased the concentration ($P < .05$) and the total excretion ($P < .01$) of chloride in the urine. However, ammonium chloride and calcium chloride resulted in a much lower incidence of urinary calculi than did sodium

chloride, and potassium chloride increased calculi formation. Feeding 4% of sodium chloride resulted in the highest concentration of chloride in the urine with only a slight reduction in calculi formation. These results support our contention that an elevated urinary excretion of chloride does not in itself offer protection against calculi formation.

The supplements providing sodium and potassium (sodium chloride and potassium chloride, respectively) significantly increased retention ($P < .01$) and urinary excretion ($P < .05$) of the respective cations. In addition sodium chloride significantly ($P < .05$) decreased the fecal excretion of potassium. While there appeared to be no relationship between urinary sodium concentration and the occurrence of urinary calculi, it cannot be concluded at this time whether the increased urinary potassium concentration may have contributed to the higher incidence of calculi in lambs fed potassium chloride.

Supplemental dietary calcium resulted in a greater absorption of both sodium and potassium as evidenced by significantly ($P < .01$) lower fecal excretion of these cations in lambs receiving either 1% calcium chloride or 2% calcium carbonate. While there was a trend toward higher urinary excretions of sodium and potassium in lambs receiving either of the two sources of supplemental calcium, the values differed significantly ($P < .05$) from the controls only for urinary sodium excretion in lambs fed calcium carbonate. However, the comparable value for lambs fed calcium chloride approached significance at the same level of probability. The possibility that higher urinary

sodium values may contribute, in part, to the protective effect of supplemental calcium appears unlikely in view of the small degree of protection provided by sodium chloride which contributed the highest urinary sodium level.

For lambs fed 4% sodium chloride and those fed 1% calcium chloride, urine volumes were approximately 2 and 1.5 times, respectively, higher than for control lambs. Variations in average urine volumes did not appear to be related to the differences in urinary calculi formation in this experiment. However, the average urine phosphorus value for lambs fed sodium chloride was comparable to that for the controls despite approximately a twofold difference in urine volume. Thus, it appears that for an increase in urine volume to be effective in aiding against phosphatic urolithiasis it may be necessary for it to be accompanied by a concomitant reduction in urinary phosphorus level.

It can be concluded from these data that variations in the urinary cations, calcium, sodium and potassium, or the anion, chloride, without a concomitant reduction in urinary pH, play no major role in the prevention of phosphatic urolithiasis by the dietary salts used in these studies. Postulations of protective effects for the various salts by modes of action other than those previously recognized, i.e., a lowering of urinary pH (Vermeulen et al., 1951; Leoschke and Elvehjem, 1954; Bushman et al., 1967), and a decrease in phosphate excretion (Gill et al., 1959; Bushman et al., 1965a, 1965b) do not appear warranted on the basis of these data.

Blood serum data are presented in table 13. There was no

TABLE 13. BLOOD SERUM VALUES, PHASE 2

Treatment	Control	1% NH_4Cl	1% $CaCl_2$	1% KCl	4% NaCl	2% $CaCO_3$
Number of lambs[a]	13	16	14	13	13	12
Serum values, mg./100 ml.[b]						
Calcium	9.49	10.07	10.88	10.49	10.08	10.17
Phosphorus	10.18	9.96	9.28	9.96	10.12	9.81
Chloride	420.9	448.8	431.5	433.5	435.2	420.6
Sodium	451.7	245.0	240.0	257.9	262.5	264.6
Potassium	24.12	22.64	22.72	26.66	29.94	22.84

[a] Initially 16 lambs per treatment, but some lambs had to be removed for failure to adapt to the metabolism cages.

[b] Samples taken at the end of the collection period.

significant treatment effect on serum calcium, phosphorus, chloride, sodium or potassium in this experiment.

Summary

A total of 216 wether lambs were used in an experiment including feeding and balance trials. The lambs were fed a high-phosphorus basal ration, known to be calculogenic, supplemented with either 1% ammonium chloride, 1% calcium chloride, 1% potassium chloride, 4% sodium chloride or 2% calcium carbonate.

During an 88-day period, the control lambs developed a 50% incidence of urinary calculi. The calculi incidence for lambs fed the various salts were ammonium chloride, 5%; calcium chloride, 16%; potassium chloride, 85%; sodium chloride, 35% and calcium carbonate, 30%. The reduction in urinary calculi incidence resulting from the feeding of ammonium chloride, and the increase from the feeding of potassium chloride were significant ($P < .05$).

Excretion and retention data dispute the existence of any protective action manifested through variations in excretion patterns of calcium, sodium, potassium or chloride unless accompanied by a concomitant reduction in urine pH.

Average weight gain was reduced significantly ($P < .05$) by the feeding of 1% potassium chloride, and a reduction accompanying the feeding of 1% ammonium chloride approached significance at the same level of probability.

SUMMARY AND CONCLUSIONS

1. High levels of dietary phosphorus, or disturbances in phosphorus metabolism resulting in an elevated level of urinary phosphorus is probably the most important measurable factor predisposing calculi development in an alkaline urine.
2. There is no difference in the effects of high dietary levels of monosodium phosphate, disodium phosphate or sodium tripolyphosphate in producing urinary calculi.
3. Dicalcium phosphate is less likely to cause calculi formation than the sodium phosphates when both are fed in rations with comparable levels of calcium and phosphorus. This is probably due to a lower availability of the phosphorus in dicalcium phosphate.
4. Increased levels of dietary calcium or magnesium tend to reduce the incidence of urinary calculi, presumably through the formation of an insoluble phosphorus precipitate in the gut lowering the absorption of phosphorus.
5. A dietary calcium-to-phosphorus ratio exceeding 2:1 is required for the prevention of urinary calculi when high levels of phosphorus are fed. An increase in the calcium-to-phosphorus ratio also appears to improve weight gains in lambs receiving a high level of dietary phosphorus.
6. Dietary magnesium offers no more or no less protection from calculi formation than a comparable level of calcium.

7. Ammonium chloride or calcium chloride can be used effectively for the prevention of phosphatic urinary calculi formation.
8. The optimum level of ammonium chloride and calcium chloride appears to be between 1.0 and 1.5% of the air-dry ration.
9. Ammonium chloride and calcium chloride appear to exert their influence through a reduction in urinary pH.
10. A protective level of ammonium chloride may reduce feed consumption and weight gain of lambs under some conditions of feeding such as inadequate mixing.
11. Sodium chloride fed at a level of 4% of the air-dry ration may not be expected to offer significant protection from urinary calculi when lambs are receiving a high-phosphorus calculogenic ration. However, this does not preclude the possibility that it may offer a high degree of protection when used with rations containing lower levels of phosphorus.
12. If an increase in urine volume resulting from the feeding of sodium chloride is to be effective in the prevention of phosphatic urinary calculi it must result in a lower concentration of phosphorus in the urine.
13. Potassium chloride fed as 1% of a high-phosphorus, calculogenic ration may increase calculi formation and lower feed consumption and weight gain.
14. Variations in urinary excretion patterns of calcium, magnesium, sodium, potassium and chloride do not appear to play a major role in the prevention of urinary calculi.

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